The Journal of Laboratory and Clinical Medicine

VOL. VI. ST. LOUIS, AUGUST, 1921 No. 11

ORIGINAL ARTICLES

AN ACCURATE METHOD FOR THE CLINICAL DETERMINATION OF EARLY ARTERIAL DISEASE*

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THE diagnosis of arterial disease must be made early if it is to be utilized to any definite practical end in clinical cases. By the older methods, early diagnosis is rarely or never possible except largely on supposition or on historical grounds. As a result very little curative effect is accomplished in cases of arteriosclerosis except in early specific cases in which, as a rule, the diagnosis of arterial disease is based upon the law of probability, rather than on actual detection of changes in the blood vessels.

Thus far early arterial disease has been detected chiefly by an ophthalmoscopic study of the retinal arterioles. This has indeed proved most valuable, but its successful employment presupposes a considerable technical skill with the ophthalmoscope and a very wide experience is necessary for accuracy. The method at its best is hardly applicable for routine use in the hands of the average clinician and yet the data which its utilization furnishes is material of the most elemental importance in the subsequent treatment of any case.

If real results are to be expected in the management of this type of disease, it must be instituted very early and as a rule, this is not possible or probable under usual diagnostic routine, or until such time as irradicable lesions have been inflicted.

It is generally supposed that the retinal arteries are among the very first to show indications of developing arterial disease and this is in all probability a correct statement, but we must recall that in very many cases, especially in arteriosclerosis, lesions are not always diffuse or universal, but are more or less limited to or more pronounced in certain systems or distributions. Even

*Presented before the Association of American Physicians, Atlantic City, N. J., May 12, 1921.

if this assumption be accepted, it still remains that the retinal vessels correctly typify those of the most primitively important centers, that is, those of the cerebrospinal axis.

The method to which I wish to call your attention was originally demonstrated to me by Dr. David Dennis of Erie, Pennsylvania, who already has made several contributions to medical literature concerning the method, chiefly in ophthalmologic journals.

It is based on the fact that the conjunctival arterioles and other blood vessels show changes quite consonant with those present in the retinae. That this is a justifiable assumption is borne out by the fact that the conjunctival arterioles originate from the anterior and long ciliary branches of the ophthalmic artery and to a lesser extent from the external and internal palpebral branches. They thus represent in a very typical way the circulatory paths within the brain, quite as much so at least as the retinal arteries which spring from the same trunk.

Study of the vessels is accomplished by the use of two very simple and easily manipulated instruments, which are usually in the pocket of the average practitioner. The ordinary pocket electric flash light of which the most convenient for this purpose is the "fountain pen" type is used for illumination. The patient is directed to turn his eyes to either the one side or the other, and the light held at a distance of about three to four cm. is directed obliquely onto the ocular conjunctiva. Study of the vessels is then made through an ordinary ophthalmologist's loupe, which is the most adaptable to the purpose, though other lenses are also fairly satisfactory. The loupe is held at the proper focal distance and for most satisfactory study the eye of the observer is brought close to the lens, just as in the use of the microscope. The vessels under study in the various levels of the membrane are brought sharply into focus by moving the lens to and fro and for the purpose of steadying it the fingers of the lens hand may be rested on the orbital arch of the patient. The study may be made in the diffuse light of the examining rooms or even more satisfactorily in the dark room. When my flash light has been too weak I have secured my lighting by the use of the ordinary head mirror and the usual examining lamp, such as one uses in the examination of the nose and throat. A sharply condensing light, such as is employed in the illumination of the sinuses and so on, is of course very desirable, but the ordinary small, electric flash is all that is needed.

One is thus able to study in a most satisfactory way the larger branches of the ciliaries and palpebrals, the tiny arborizing arterioles and even the capillary circulation in the ocular conjunctivae as well as the return circulation in the venules and the larger ciliary and palpebral branches of the ophthalmic. These vessels are displayed, as it were, beautifully spread out on an ideal pearly white background which forms a most pleasing contrast to the yellow red of the arterioles and the deeper carmine color of the venules. The arrangement for study is as definite and satisfactory as is possible in a well-stained and technically perfect microscopic slide, and by slight variations in focus a vessel may be followed throughout its entire arborization. The method is much more satisfactory than the study of the retinal circulation

because of the sharp contrast of the white background as compared to the pinkish tinge of the retina and it is much more representative of the internal cerebral circulatory condition than the tiny retinal artery since we have here represented for our study four systems of arteries all derived from the same branch of the internal carotid, namely from the ophthalmic. A further great advantage over the study of the retinal artery is represented in that we are here able to observe in the conjunctival circulation the effects of arterial disease on the adjacent tissue. This is particularly fascinating in cases of arteriocapillary fibrosis, in calcifications, fatty degenerations and in instances of hypertension of marked degree. A disadvantage of the method lies in the fact that in cases in which disease of the conjunctiva is present, local vascular lesions are found, particularly in conjunctivitis and in the membrane of patients who have been exposed to the traumatism of wind, intense light, and of irritating chemicals and gases.

The studies of Dr. Dennis of Erie and of Dr. Reese of New York have shown quite finally that these changes in the conjuctival vessels are entirely comparable in any given instance with the alterations manifest in the retinal arteries; this I have also corroborated within the past four years that I have been using the method as a routine, insofar as my skill with the ophthalmoscope may be trusted.

The great advantage to the clinician in the method is that a highly sufficient technical skill may be acquired with a few days' practice. It demands no special instruments and less time is required for the intimate study of the minute circulatory changes in the cerebral vessels than is necessary for a reasonably careful palpation of the radial, brachial or temporal arteries. There is no longer any excuse for mere speculation as to the condition of the blood vessels of the brain, from 30 to 120 seconds of time is sufficient to give one a very satisfactory and entirely accurate knowledge of such changes, if present.

I have found the study most helpful and of tremendous advantage in the detection of the earlier changes present as in syphilis and arteritis from any cause. I have become particularly interested in the study of the terminal arteriole changes of hypertension.

One of the pitfalls into which you will sooner or later fall if you follow this method is the employment of the corneal microscope, by means of which the most intimate study may be made, not only of the microscopic changes in the walls of the arterioles, capillaries and venules, but also the physiologic and pathologic variations in the circulating stream can be made out with greater lucidity than is possible in the web of the frog's foot under low powers of the microscope. This requires, and I can abundantly testify, very considerable degree of technical skill, costly and delicate instruments and most expensive of all, a very large amount of time and patience. This means a study of the conjunctival circulation, however, is indispensable to check up as it were, the gross pathology observable with the hand lens. However, the short distance which I have already traveled into the art shows me that it offers a most valuable method for the microscopic study of the circulatory conditions of the brain. This is transferable by inference to the other parts of the body in gen-

eral arterial disease. It is a study which bids fair to be of even greater value to the internist than to the ophthalmologist.

The most signal benefit conferred by the methods presented is, of course, the ready detection of the very early changes in the arterioles, which may lead on to generalized arterial lesion that plays so important a rôle in the evolution of many internal diseases. By these studies one may detect sufficiently early changes in the arterial system to permit him to inaugurate modifications in habits of life, exercise, diet, etc., also to render possible, in association with proper medication, the introduction of a treatment sufficiently early so that real preventive measures are attainable in many cases.

I have used the study of the conjunctival vessels as a routine in my physical examination of office cases and to a considerable degree also on my hospital services now for about three years. I have been able to satisfy myself that the conjunctival vessels very satisfactorily represent the existence and type of a general arteriosclerosis as confirmed finally by the usual methods of study.

Changes detectable in the conjunctival vessels admit of practically the same classification which we recognize pathologically in our study of anatomical material and a little technical experience readily permits the early recognition of these changes as it were "in vivo."

I wish to present here very briefly a study which I have made of the conjunctival vessels in cases of hypertension. One of the most striking changes which the observer notes in cases of hypertension is the elevation of the more superficial vessels above the surface of the conjunctiva, so that when the light is directed diagonally on the surface of the conjunctiva a reticular framework made up of the tense blood vessels is shown. The arterioles show in early phases also a clear sharp line of demarcation, sharply differentiated off from the surrounding tissues as though overtensely filled. This is manifest even when there are extensive degenerative, infiltratory or inflammatory alterations in the surrounding tissues.

Many of the more superficial vessels are thrown into numerous convolutions as is seen grossly in the contorted arteries and the calcifications of old age. It must be noted, however, that the venules of the conjunctivæ are often markedly convoluted and that this is apparently a normal condition in certain persons, for I have observed its frequent presence in the conjunctival veins of infants and youths entirely independent of any apparent circulatory disease. This is not, however, true of the arteries in which convolutions apparently bespeak only a disease process associated with arteriosclerotic changes or with hypertension.

When a period of hypertension of long standing has been succeeded by a hypotension as from a circulatory decompensation, this elevation and contortion of the arterioles is still preserved and may serve as a sign apparently that a hypertensive condition has preexisted.

Another very frequent, but by no means constant finding in the conjunctival vessels in hypertensive cases is the existence of nodes of lumen contraction so that though the vessel walls are represented by a relatively broad yel-

lowish or whitish streak, but a mere trickle of golden red represents the contracted lumen, at the proximal end of which a minute dilatation of caliber is demonstrable. This is particularly evident in very long standing cases and it apparently represents an arteritis or arteriofibrosis with obliteration of the lumen. The extreme stage of this alteration is represented by complete obliteration so that vessels are represented merely by yellowish or grayish cord-like structures. Where many of these obliterated vessels are present in the conjunctiva, the whole membrane is given a grayish or yellowish white color so that an appearance of extreme anemia may be presented while the blood picture shows a normal condition of affairs quite surprising to the clinician who is accustomed to judge as to the degree of anemia from the color imparted by the conjunctival vessels. This statement comprehends the palpebral as well as the ocular conjunctiva.

The first two signs of hypertension appear relatively early in cases of hypertension, the latter only in the long standing cases.

Tiny aneurysmal dilatations of the conjunctival arterioles are not commonly seen in hypertensive cases. They are, as Dennis has stated to be the case, most common in instances of true arteritis where local changes in or about the arterioles predominate but, of course, they may on theoretical grounds be expected in those cases where an arteriocapillary fibrosis is the cause of the hypertension.

VENTILATION, WEATHER, AND THE COMMON COLD*

A Study of the Prevalence of Respiratory Affections Among School Children and Their Association with School Ventilation and the Seasonal Changes in Weather

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INTRODUCTORY

DURING the last twenty years there has been a great amount of experimental work on ventilation and its effect on the body. Workers in Europe and the United States are in substantial agreement that it is the thermal factors—temperature, air motion and humidity—which exercise the greatest influence on human comfort, health and efficiency. The chemical composition of the air we ordinarily breathe—leaving out of consideration for the moment those special industrial problems involving gases, fumes and dusts—is of relatively little moment in its effect on human conduct. School children are far better off in a cool, airy room, regardless of the carbon dioxide content of the air, than they are in air virgin pure chemically which is overheated.

It is most important that the facts as we have stated them should be clearly understood, for otherwise, there is bound to arise, as there has in the past, a misunderstanding as to the suitability of different methods of ventilating school buildings.

If variable, as opposed to uniform, temperature, air motion and humidity are desirable factors, then very satisfactory conditions can be maintained in school rooms by ventilating with the windows, protected by deflectors, and an exhaust duct on the opposite side of the room, heating being by direct radiation beneath the windows. This method of ventilating will not always give good aeration. At times the room will be amply flushed with outside air. At other times, due to shifting winds, the circulation will be lessened, the room will not be thoroughly flushed, and the carbon dioxide content will rise, indicating an accumulation of the products of exhalation and body vaporization. Even though the aeration of the room fluctuates, it is possible to have coolness at all times and variability, and if the room is cool and

*This is an abridged form of a dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Public Health at the University of Michigan, 1920.

This study was conducted jointly by the Bureau of Child Hygiene of the New York City Department of Health, represented by Dr. S. Josephine Baker, Chief of the Bureau, and the New York State Commission on Ventilation, represented by the author, who then held the position of chief of the investigating staff. The collection of sickness records and the taking of temperature and other observations on air conditions was done by nurses and physicians of the Health Department under the supervision of Drs. L. Marcus and R. H. Willis. The routine clerical work of tabulation was likewise conducted by the Health Department under the immediate direction of Dr. Franklin Van Wart. The planning of the investigation, selection of schools, initial instruction of the field staff and the final analysis and interpretation of results is largely the work of the author.

variable within certain limits it makes little practical difference as to the humidity.

On the other hand, if the experimental data of the last two decades are wrong in minimizing the relative value of chemical purity of the atmosphere, then window ventilation as we have described it is inadequate, and it will be necessary to insure at all times voluminous and continuous flushing of the room with outside air. This can be done only by mechanical means, that is by plenum fans or blowers.

There has existed for some time a controversy as to the relative merits of natural and mechanical ventilation. An inheritance from the days of Pettenkofer, when chemical purity was regarded as vital, has kept alive the carbon dioxide content as the standard of ventilation goodness. This standard persists to this day. Measured in these terms the window ventilated room falls into disrepute. A carbon dioxide standard of 6 to 10 parts per 10,000 automatically throws any form of window ventilation into the discard. It is only by mechanical means that this degree of chemical purity can be at all times assured.

With the development of mechanical ventilation there has grown up an impression that uniformity in temperature, in air motion and in humidity is ideal, and again the mechanically forced ventilation far excells the gravity method in this respect. Furthermore the dust in outside air can be removed readily under the mechanical system by the introduction of air washers. This is not possible with window ventilation.

There is much then that can be accomplished with the plenum system that is not possible under the window method. The question arises as to whether the superiority of the mechanical system is superficial, a matter of a relatively unimportant refinement so far as the school classroom is concerned. The drawbacks to the mechanically ventilated classroom are its tendency to overheating, its unstimulating uniformity and its greater expense.

There is much to be said on both sides. The advocates of window ventilation are impressed by its success with tuberculously inclined and undernourished children. It has a wide application in our public schools at the present time. If good for sick children, why not for well children? Is window ventilation in the schoolroom to be ruled out of consideration merely because it fails to live up to the carbon dioxide standard? It was for the purpose of testing out these principles on a practical scale that the present experiment was undertaken. After all, the proof of the pudding is in the eating. If the health of school children, as measured by the amount of respiratory illness, such as colds, tonsillitis, etc., is better under the more elaborate systems of mechanical ventilation, then let us proceed to equip our buildings in this manner. If, on the other hand, the mechanical ventilating equipment does not supply substantial benefits to the health, comfort or efficiency of our school child population, or is actually inferior in the things that count for health, let us face the matter squarely.

There are innumerable demands for public funds. If we are paying out vast sums of money for benefits which are not real but imaginary, recognition

of this fact cannot come too soon. If window ventilation provides the important essentials of a healthy atmosphere in a way that cannot be attained or improved upon by mechanical systems of indirect ventilation, then our school buildings should be built accordingly, and we should not hesitate because a worn out standard of ventilation dictates otherwise.

During the latter part of 1916, from February 14th to April 6th (8 weeks), and the winter of 1916-17, from October 30 to January 26 (12 weeks), observations were made on the health of 5500 New York City school children who were exposed to various types of ventilation in 12 different school buildings. In general these ventilation systems may be classified under three main headings, as follows:

A—Cold, open window rooms, gravity exhaust.

B—Cool, window ventilated rooms, gravity exhaust.

C—Plenum, fan ventilated rooms with gravity exhaust and with windows closed.

As an index of health, the sickness records of the pupils were used. The condition of the air was determined by readings of temperature and humidity and by the personal sensations of the observers as to temperature, moisture, air motion and odor. It would have been desirable to determine also the carbon dioxide content of the air, but this involved analytical work which the staff was unable to do. The absence of these latter data was not serious, however, for the Ventilation Commission had available a mass of data on this subject collected over two years' time, and it was well established that the carbon dioxide content of fan ventilated rooms averages several parts lower than in window ventilated rooms.

For the convenience of the reader we shall reserve the description of working methods and ventilation types in individual schools for the later pages and shall proceed with an account of the findings of this study.

RESULTS OF SICKNESS SURVEY IN DIFFERENT TYPES OF VENTILATED SCHOOL ROOMS

The first half of the study in the spring of 1916 covered 2500 pupils in 58 classrooms distributed among 8 schools. The second half in the winter of 1916-17 was represented by 3000 pupils in 76 classrooms in 12 schools.

In both studies the absences due to respiratory illness and the respiratory illness among pupils present in school was greatest in the fan ventilated rooms, Type C. This is the result after combining all records and disregarding in this instance the balancing of the type of pupil, location of school, etc., which will be treated more at length later on.

The excess of respiratory illness in the Type C rooms holds good both for absentees and those in school. The total illness is least in the second group, or cool, window ventilated rooms. The difference, however, between the first and second types of window ventilation is less than between either the first or second and the third. In other words, assuming for the moment that these differences are due to atmospheric influences, the air conditions in the first two types do not produce greatly divergent effects, but the influences at work in the third type are distinctly less favorable.

The significance of these two sets of results may be expressed in this manner; for every 100 cases of respiratory illness in the cool window ventilated rooms, there are 152 in cold, window ventilated rooms, and 231 in fan ventilated rooms.

TABLE I

RESPIRATORY ILLNESS PER 1000 REGISTRATION (PUPIL-SESSION) UNITS*

Ventilation Type	FIRST STUDY Among Absentees	FIRST STUDY Among Pupils in School	FIRST STUDY Total	SECOND STUDY Among Absentees	SECOND STUDY Among Pupils in School	SECOND STUDY Total
A-Cold, Window Rooms	10.6	37.2	47.8	9.2	75.3	84.5
B-Cool, Window Rooms	10.2	22.1	32.3	10.7	44.1	54.8
C-Fan Ventilated	14.2	76.0	90.2	13.0	98.4	111.4

TABLE II

BASIC FIGURES FROM WHICH RATES IN TABLE I ARE COMPUTED

Ventilation Type	FIRST STUDY Total Registration Units	FIRST STUDY Total Absence Units due to Respiratory Illness	FIRST STUDY Total Units of Respiratory Illness among Pupils in Attendance	SECOND STUDY Total Registration Units	SECOND STUDY Total Absence Units due to Respiratory Illness	SECOND STUDY Total Units of Respiratory Illness among Pupils in Attendance
A	61,658	655	2,298	89,067	822	6,705
B	71,231	728	1,578	113,959	1,218	4,661
C	65,088	925	4,950	115,215	1,497	11,329

The actual temperature conditions found in the three types of rooms are disclosed in the two tables following, in one of which results are expressed as averages, and in the other by temperature groups.

TABLE III

AVERAGE OF ROOM TEMPERATURES

Ventilation Type	First Study	Second Study	Mean
A	58.8	59.1	59.0
B	66.9	65.9	66.4
C	68.8	67.9	68.4

*The unit of illness was one pupil per half-day school session. Illness is reported in pupil session units. One pupil ill ten sessions counted the same as ten pupils ill one session. A pupil was continued on the register regardless of the length of absence from school unless it was found that the family had moved away from the school district, or that the pupil had left school permanently.

Separate tabulations have been made of illness resulting in absence from school and illness among pupils who continued to attend school. The absences are classified as (1) absence from respiratory illness, including coryza, bronchitis, pharyngitis, laryngitis, tonsillitis, pneumonia, tuberculosis and a miscellaneous group variously termed grippe, colds, sore throat, etc.; (2) absence from illness other than respiratory, namely: stomachache, backache, broken leg, etc. In this group were also the acute infectious diseases such as diphtheria, measles, scarlet fever, whooping cough, chickenpox, mumps, etc. This was done because it was felt that the spread of these infections was largely determined by specific susceptibility rather than by atmospheric influences; (3) absence due to causes other than illness, such as staying at home to mind the baby, going on a visit, shopping with parents, truancy, etc.

The fan ventilated or Type C rooms averaged but two degrees higher than those of Type B and nearly ten degrees warmer than Type A. Type A was extremely cold for a school room. Type B was cooler than is customarily found. The average temperature of Type C, though higher than B, was not exceptionally high.

There was a wide range in the daily temperatures, which are lost sight of in the averages.

TABLE IV

FREQUENCY DISTRIBUTION OF TEMPERATURES
PER CENT OF SESSIONS

Types Ventilation	59° and below	60-69°	70° and over
	1st Study		
A	56	41	3
B	5	61	34
C	4	57	39
	2nd Study		
A	46	48	6
B	8	88	4
C	0.2	99	0.8

The A rooms rarely reached 70°. Fully half of the sessions were below 59°. The B and C rooms rarely fell below 60°. The temperatures in the second study were much more uniform, particularly as regards Type C and to a lesser extent Type B. More than one-third of the sessions in B and C in the first study were above 70°. Much of this represented an overheated condition. However, the first study was made in the late winter, when greater outdoor variation is experienced than from November to January. Although the proportion of sessions between 60 and 70° does not appear to differ much in the B and C rooms, yet there was an appreciable difference within this range. Thus, in the first study 24 per cent of sessions in B were from 60° to 64°, whereas but 6 per cent of the sessions in C were within this range. Thirty-seven per cent of B sessions were from 65 to 69 as against 51 per cent of the C sessions. The C rooms were warmer, as the averages have already indicated.

The interesting point to be noted is that whereas there was but two degrees difference in temperature between the B and C rooms, there was a wide difference in the sickness rates. Between 7 and 8 degrees separated Types A and B, and yet in spite of this the sickness rates were quite similar. Evidently some factor other than temperature operated differently on these three classes of rooms or else a rise of two degrees above 66° is far more conducive to colds than a drop of as much as 7 degrees.

HUMIDITY

The relative humidity ranged from 38 to 46% and did not differ greatly in the three classes.

As would be expected, the relative humidity was higher in the colder rooms. With the same amount of moisture present as in Type A, the relative humidity in the first study at the temperature in Type B would be 33.4,

TABLE V

RELATIVE HUMIDITY

Ventilation Type	First Study	Second Study
A	43.7	46.3
B	37.8	43.0
C	37.8	41.2

and in Type C 31.4. The corresponding figures in the second study would be 29.9 for B and 28.7 for C. It would appear, therefore, that there was an accumulation of moisture in the Type B rooms, and this could only come from reduced aeration. In Type C the same explanation would hold. The rooms at P. S. 59 were humidified, as were also the rooms at P. S. 51 and 97, but this would hardly affect the average of all rooms in the group to this extent. It is also possible that the taking of the humidity reading was not as accurately done in the dry rooms, the wet bulb being read before the mercury column had completed its fall. These readings are higher than we should expect at this season of the year, judging from records taken by the Commission in similar rooms in other schools.

Other indices of air conditions were the opinions of the nurses who visited the rooms at least twice daily.

FRESHNESS AND ODOR

The results of the nurses' votes on the freshness or lack of freshness and presence of odor in the rooms are given in Table VI.

TABLE VI

Ventilation Type	PERCENTAGE OF SESSIONS JUDGED		
	Exceptionally Fresh	Odor Absent but not Exceptionally fresh	Odorous
	First Study		
A	62	24	14
B	25	57	18
C	21	64	15
	Second Study		
A	69	28	3.2
B	18	62	20
C	22	67	11

The results are very interesting from several points of view. The Type B rooms are most odorous in both studies. The excess over the others was slight in the first study, 18, as compared to 15 for Type C and 14 for A. It was more marked in the second study, 20, as against 11 for C and only 3.2 for A.

The freshest rooms are the coldest rooms. Sixty-two per cent of the sessions in Type A in the first study were judged exceptionally fresh, and in the second study the figure was 69 per cent. The figures for Type B were 25 per cent in the first and 18 in the second. Type C had 21 per cent in the first and 22 per cent in the second.

What seems clear from these figures is that to be exceptionally fresh the greater part of the time, rooms must be well below 65 degrees in temperature. Warmer rooms may be free from odor and yet exceptionally fresh not much over one-fifth of the time.

In rooms that did not differ greatly in temperature, as B and C, the greater aeration produced by fan ventilation reduced odor to a slight degree in the first study, and to a marked degree in the second. It failed to make the rooms any fresher in the first study, but did help out in the second study.

Although we do not possess complete records of the carbon dioxide content, there is little doubt but that the smallest amount would be found in the fan ventilated rooms, Type C, as we have already pointed out. Repeated records collected in three schools show this tendency (Table VII).

TABLE VII

CARBON DIOXIDE IN PARTS PER 10,000

SCHOOL	VENTILATION TYPE B	C
33	6.9	5.5
115	7.6	6.5
97	8.6	5.7

The markedly greater freshness of the Type A rooms is due, without question, to their low temperature, and not because of the greater chemical purity of the air. Freshness is not a question of odor, for the Type B rooms were fresher than C and yet more odorous.

SENSATION OF TEMPERATURE

The recorded votes of the nurses as to whether the temperature of the rooms felt "too cool," "satisfactory" or "too warm" are given in Table VIII.

TABLE VIII

VENTILATION TYPE	PER CENT OF SESSIONS JUDGED TOO COOL	SATISFACTORY	TOO WARM
	First Study		
A	7.9	77	16
B	6.2	78	16
C	10	76	14
	Second Study		
A	26	70	3.9
B	8.6	80	11
C	3.6	85	12

In the first study the per cent of satisfactory sessions was about the same in all three types, being in the neighborhood of 77 per cent. There were more sessions judged "too warm" in Types A and B than in C, although the average temperature was lower than C. The "too cool" sessions were most numerous in Type C, the rooms of highest temperature. In other words, the coldest rooms felt warmer than the warm rooms. In view of the actual temperature found, it would appear that the nurses were influenced in their judgment of what the

temperature should have been rather than by actual sensation. In no other way can we account for the votes in rooms whose temperature differed by at least ten degrees, as was the case in Types A and C.

The votes in the second study more nearly reveal the actual temperature condition as indicated by the thermometer. In A 26 per cent of the sessions were judged "too cool." In fact, 4 per cent were voted "too cold"—an extreme condition. In Type B, 8.6 per cent were "too cool" and in C 3.6 per cent.

Type C had the greatest number of sessions judged satisfactory as to temperature; namely, 85 per cent. The corresponding figure for Type B was 80 and for A, 70.

Too great warmth was experienced 12 per cent of the time in C, 11 per cent in B and but 3.9 per cent in A.

The sensation of temperature reflects the actual thermometer reading—to some extent at least. There was much lesss overheating in the second study, and this agrees with the lesser number of "too warm" votes.

SENSATION OF MOISTURE

As will be seen from the figures given below, over 80 per cent of sessions in all three types of the first study were judged satisfactory as to moisture. Both moisture and dryness were most pronounced in Type C.

TABLE IX

VENTILATION TYPE	PER CENT OF SESSIONS JUDGED MOIST	SATISFACTORY	DRY
	First Study		
A	5.9	88	6.7
B	1.7	89	9.0
C	7.3	83	9.7
	Second Study		
A	33	66	0.6
B	20	74	6.4
C	8.5	84	7.9

The second study reveals a greater divergence between the rooms. Type C had the highest percentage of satisfactory sessions, 84. Type B had but 74 per cent and Type A, only 66. The cooler sessions are associated with moisture, the warmer sessions with dryness. One-third of all sessions in A were moist and less than 1 per cent dry. Twenty per cent of sessions in B were moist and 6.4 per cent dry. Eight and five-tenths per cent of C were moist and 7.9 per cent dry.

There was less overheating in the second study and the sensation of dryness is less. Type C is similar in both studies. The other two types are different in that the second study shows many more moist sessions.

AIR MOTION

The greatest proportion of satisfactory votes as to air motion was found in A. Moving air was noticed most frequently in A and least in B. The results

of both studies are similar, although it is surprising to find the sessions in Type C judged "dead" to be more numerous in the second study where the air flow through the rooms was greater and the temperature was lower.

TABLE X

PER CENT OF SESSIONS JUDGED

VENTILATION TYPE	DEAD	SATISFACTORY	BREEZY
	First Study		
A	4.7	81	14
B	15	78	7.1
C	9.3	73	18
	Second Study		
A	9.2	74	17
B	18	75	7.0
C	23	68	9.8

(To be continued.)

A REVIEW OF NINETY-FOUR NECROPSIES. WITH SPECIAL REFERENCE TO THE PNEUMONIAS*

By Geo. W. Covey, A.M., M.D., Lincoln, Nebr.

INTRODUCTION

UNFORTUNATELY the pathologic service in our center overseas was undermanned and underequipped, and the work had to be done under very harassing conditions. In a period of less than five months more than 30,000 patients passed through this center, of whom 429 died. Of these, 80.3 per cent were autopsied—a total of 356 necropsies. The greater part of these were done by my chief, Dr. Moses Barron, pathologist of the University of Minnesota, and by myself. More than one-half of the deaths occurred in one month—October, 1918, the daily average being eight. In addition, Dr. Barron was chief of the laboratory service, and I examined more than a thousand tissues microscopically during this time. Of the 106 postmortems done by me, I have complete records of the gross findings in 94.

A very complete and cleverly written résumé of the 356 cases has been published in the *Archives of Internal Medicine* for September, 1919, by Dr. Barron. In this he has touched on most of the important pathologic lesions that came under our observation. My excuse, therefore, for a paper on my own records is to make a somewhat more detailed study of a few points concerning the heart, aorta, kidneys, and sinuses, and to dwell especially on the pneumonias which we encountered.

HEART

One of the most striking features of our heart findings was the paucity of chronic lesions. In other words, the heart boards had efficiently ruled out chronic diseases in this field.

I have here recorded the weight of each of 61 hearts (Table I). The min-

Table I

Number of hearts—weights recorded	61
Average weight heart: males—Morris-Grey	341 grams
Average weight hearts in this series	331.7 "
Maximum weight	450 "
Minimum weight	220 "
Number weighing more than the average	29 "

imum was 220 grams, the maximum 450 grams. Eleven hearts weighed 400 grams or more. Twenty-nine weighed more than 340 grams. The average

*Read before the Lancaster County Medical Society, April, 1920.

weight was 331.7 grams. The average weight of hearts in the male recorded by Morris and by Grey is 341 grams. We see, therefore, that our general average falls about 10 grams low, but that 29, or 47.5 per cent, were above the average.

Seven hearts, or 7.4 per cent, showed a patent foramen ovale (Table II).

TABLE II

Patent foramen ovale	7	or 7.4	per cent
Number hearts with valvular lesion	11	or 10.3	" "
Number hearts with acute valvular endocarditis	4	or 3.7	" "
Number hearts with acute endocarditis, mitral alone	1	or 0.9	" "
Number hearts with acute endocarditis, mitral and tricuspid	1	or 0.9	" "
Number hearts with acute endocarditis, mitral and chronic elsewhere	2	or 1.8	" "
Number hearts with acute endocarditis, aortic	0		

Eleven of the 94 hearts, or 10.3 per cent, showed valvular lesions. Only four of these were acute infections. The chronic lesions were universally of a mild and almost insignificant degree. The mitral valve was affected in five cases (4.7 per cent), four of which were acute. Only one of these was an acute lesion of the mitral valve alone; one occurred with an acute endocarditis of the tricuspid valve, and two with chronic disease of the aortic valve. No acute lesions were seen affecting the aortic valve, but it had some slight chronic affection in eight cases.

The myocardium showed some evidence of degeneration, such as a brownish color and flabby consistency in eight cases, or 7.5 per cent. None of these was very marked, and no acute infectious process was seen here. Dilatation is recorded in nine cases, or 14.7 per cent.

Twenty-five cases, or 23.5 per cent, showed some lesion of the pericardium or epicardium. Five of these were acute, three being a real acute pericarditis and two subpericardial hemorrhages. The remainder consisted of some remnant of past disease of the heart coverings, the most interesting of which were the so-called "soldier-spots." A true soldier-spot is a thickened area of epicardium corresponding to a complemental "spot" on the pericardium, varying from a few millimeters to 1½ cm. or more in size. Some were found in which a fibrous band, running from epicardium to pericardium, connected these two "spots." In others the band had apparently gone further, the fibrous brush of tissue being absorbed and leaving a coin-like whitish thickening on the two membranes. Six of these cases, or 5.64 per cent, showed typical "soldier-spots," while 13 others showed smaller or larger areas of thickened epicardium alone.

AORTA

Five aortas had evidence of aortitis, nonluetic in character, marked enough to be considered abnormal; yet none of them was remarkable in extent or degree. Three cases of true syphilitic aortitis, each with aneurysm, were posted in the Center, and all of them happened to fall in my own service. Only one of these was suspected prior to death. This was a large saccular aneurysm beginning at

the aortic ring and involving practically the ascending limb of the arch. Death occurred here due to sudden dilatation of the aortic ring. The two others were typically luetic throughout, but the aneurysm occurred in the abdominal portion. In one of these, death was due to rupture and infiltration of the retroperitoneal tissues with blood. An interesting angle of this case is the fact that a tumor of the right kidney was diagnosed. The abdomen was opened and explored by the eye and finger of the surgeon, who decided the tumor was too extensive for removal and contented himself with abducting the man's appendix. Even then it remained for the pathologist to show that the tumor behind the right kidney was blood from the aneurysmal sac. The third was entirely unsuspected and was found in a man who died of septicemia. It was a sac lying between the aorta and the vertebrae in the lumbar region. It had eroded a portion of the bodies of two vertebrae, but had caused no symptoms.

KIDNEY (SEE TABLE III)

In 69 cases the average weight of the left kidney is 186 grams; that of the right is 184. The minimum weight is 120 grams and the maximum 300 grams.

TABLE III

Number kidneys weighed—pairs	69	
Average weight kidneys in male—Kelly and Burnham	168	grams
Minimum weight kidneys in male—Kelly and Burnham	107	"
Maximum weight kidneys in male—Kelly and Burnham	264	"
Average weight left kidney, this series	186	"
Average weight right kidney, this series	184	"
Minimum weight kidney, this series	120	"
Maximum weight kidney, this series	300	"
Congenital abnormalities	2 or 1.8	per cent
Acute nephritis	28 or 26.3	" "
Chronic nephritis	14 or 13.2	" "
Nephrolithiasis	2 or 1.8	" "
Acute pyelitis and hydronephrosis	1 or .9	" "
Pyelonephritis	1 or .9	" "
Cystic degeneration	1 or .9	" "

Two cases had but one kidney each, one being a horseshoe kidney weighing 500 grams and having two pelves and two ureters, the other being a single right kidney weighing 300 grams with aplasia of the left kidney and ureter. According to Kelly and Burnham (Diseases of the Kidneys, Ureters and Bladder, Vol. I, p. 137) the normal weight of a kidney in the state of physiologic distention is 168 grams—average; the minimum 107 and maximum 264 grams. The right usually weighs slightly less than the left.

In 28 cases the kidneys showed acute nephritis; however, most of these were mild, only two being severe and the cause of death. In 14 cases mild chronic nephritis was present.

In addition, calculi were found in two cases; septic infarcts with abscess formation in two cases; acute pyelitis in two cases—one having an acute pyelitis on one side with a hydronephrosis on the opposite side; pyelonephritis in one case, and cystic degeneration in one case.

SINUSES OF SKULL, MASTOIDS AND MIDDLE EAR (SEE TABLE IV)

TABLE IV

Purulent sinusitis	37 or 34.8 per cent
Single sinus or pair	31 or 29.3 " "
More than one pair	6 or 5.6 " "
Pan sinusitis	2 or 1.8 " "
Sphenoidals	34
Frontals	5
Ethmoidals	4
Middle ear	7
Mastoid	2

Purulent sinusitis was present in 37 cases, or 34.8 per cent, a single sinus or pair being infected in 31 cases and more than one pair in six cases. All the sinuses were infected in each of two cases. The sphenoidals were involved 34 times, the frontals 5 times, the ethmoidals 4 times, the middle ear 7 times, and the mastoids 2 times.

THE PNEUMONIAS

Fig. 1.—Photomicrograph from a section of lung showing bronchopneumonia. Shows a bronchiole filled with pus. The contiguous alveoli only are filled with exudate. *1*. Lumen of bronchiole. *2*. Wall of bronchiole. *3*. Alveolus filled with exudate.

Fig. 2.—Section of typical lobar pneumonia. Note thin alveolar walls and abundance of exudate in the alveoli.

Most (about 60 per cent) of the cases showing sinus involvement were pneumonias, and a large percentage occurred during the month of October, 1918, together with the first days of November. The organisms found in the sinuses were usually identical with those from the lung or blood stream.

Of the 94 cases under discussion, 57, or 60.6 per cent, showed pneumonia. Of these, 50, or 87.7 per cent, were of the bronchogenic type; two, or 1.8 per cent, were lobar and bronchial; five, or 9 per cent, were classified as interstitial.

The most common complications of these were pleuritis and sinusitis. There

was fibrinous or serofibrinous pleurisy in 22, empyema in 10, and sinusitis, either single or multiple or pan sinusitis, in 21.

I have records of the bacteria found in 32 cases. The pneumococcus was found 14 times, the streptococcus 18 times, B. Welchii twice, and B. pyocyaneus once. In several cases more than one organism was present.

Influenza was the initial diagnosis in nine, or 16.2 per cent, of these cases.

Seventy-five per cent of the bronchopneumonias were bilateral, many being so massive that practically whole lobes or complete lungs were solid.

Among these cases there was none of pure ordinary lobar pneumonia.

I do not need to discuss the pathology of the ordinary lobar pneumonia except to call to your minds for comparison the principal points. Bronchopneumonia (Fig. 1), as you remember, has its origin in the finer radicles of the bronchi and spreads outward into the surrounding parenchyma. The "lobular" idea is slightly misleading. In sections from various parts of these infected lungs we can trace the different steps in the pathologic process. Bronchioles can be seen containing exudate and having more or less degeneration and desquamation of the cells of the mucous membrane, with congestion and dilatation of the capillaries but no surrounding inflammation. Others can be found about which perhaps but one or at most a few concentric layers of alveoli contain exudate (Fig. 1). From this we pass to bronchioles having a more or less definite concentric area of consolidation involving a narrower or wider area of parenchyma. This area is always surrounded by a zone of atelectatic alveoli, due to pressure of the distended consolidated portion, and this in turn by an area of emphysematous alveoli—probably an effort on the part of the body to compensate for the functional loss of lung tissue. These areas spread ever outward until they may meet, become confluent, and give a massive consolidation.

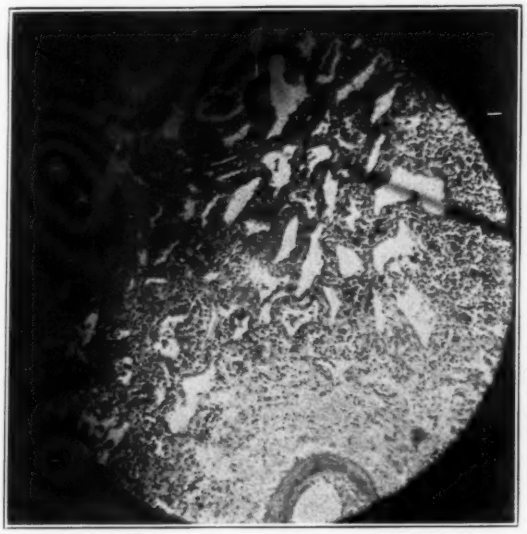

Fig. 3.—Photomicrograph of section of lung showing marked interstitial pneumonia. Note the extreme thickness and cellularity of the alveolar walls and the scarcity of exudate in the alveoli.

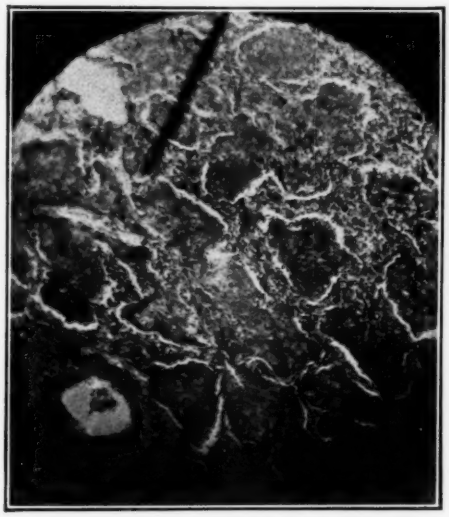

Fig. 4.—Photomicrograph of section of lung showing interstitial pneumonia. This is very similar to Fig. 3 except that the alveoli are filled with fibrinous exudate containing very few cells.

The old text book picture of mononuclear cells with no fibrin in the bronchopneumonias and polymorphonuclear cells, with much fibrin in the lobar pneumonias, must be largely overdrawn, especially as regards the fibrin deposit, for in our studies we found many bronchogenic pneumonias having as great or greater amounts of fibrin than the lobars; this more particularly in cases having been gassed, but also in others.

In lobar pneumonia (Fig. 2) the greater part or the whole of one lobe or many lobes becomes involved at once. There is congestion followed by the pouring out into the alveolar spaces of an exudate rich in red cells. In a few hours (or, at most, days) these become broken up and are gradually replaced by white cells, largely of the polymorphonuclear type. Lysis of the exudate begins, the bronchioles then become flooded with pus, the cells of which are rapidly undergoing degeneration, and the lung returns gradually to its normal state with

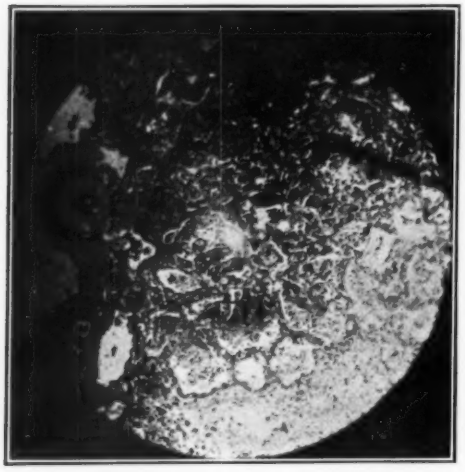

Fig. 5.—Photomicrograph of section of lung showing interstitial pneumonia. Here organization has begun. Exudate contains many fibroblasts originating in alveolar walls. Many alveoli collapsed.

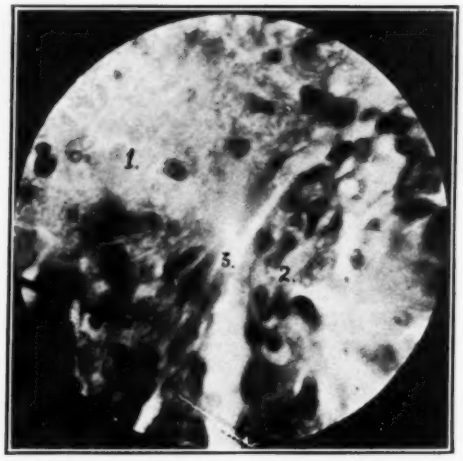

Fig. 6.—Photomicrography oil immersion of portion of section in Fig. 5 contained in the square. *1.* Exudate in alveolus. *2.* Wall of alveolus. *3.* Fibroblasts apparently originating in wall.

nothing to denote that it has been infected. We have then the stages of congestion, red hepatization, gray hepatization, resolution, and recovery.

Early in our work we encountered a relatively large number of unusual pneumonias which at first we were at a loss to name. The involvement was either lobar or bronchial. The area was airless and firm, but had a very different feel than usual. It was not quite so firm as a lobar, and not friable, but of a rubbery resiliency, it being difficult to press the finger into it hard enough to tear it, and on being released from pressure it immediately returned to its normal contour. The pleural surface was often covered by fibrinous exudate. It was deep red in color, and there were frequently subpleural pinpoint hemorrhages. The cut surface is dark red in color or may have brownish areas and occasionally small flecks of yellow. The surface is somewhat moist, but on pressure a very little fluid, not of a purulent character, is expressed. When scraped dry, a universal fine, lacy appearance results.

When these lungs were studied microscopically we found the reason for the gross appearance (Figs. 3-6). We also found that other pneumonias, bronchial or lobar, frequently showed similar small scattered areas, and that occasionally we were deceived in the gross specimens by other lesions, such as edema of the alveolar walls. Microscopically, the striking feature of these sections was a relatively immense thickening of the alveolar walls, with very little exudate in the alveoli themselves (Fig. 3). This it is that gives the characteristic gross appearance described above. In the more acute types there is more alveolar exudate than in the more chronic. This consists of serofibrinous material with scattered cells, a few of which are polymorphonuclear leucocytes, the remainder mononuclear cells, lymphocytes and desquamated endothelium (Fig. 5).

The alveolar walls are edematous and their capillaries are dilated and injected with red cells, but the striking feature is their marked cellular infiltration (Fig. 3). They are often crowded full of cells, at times polymorphonuclear, with scattered large mononuclear cells; again, almost entirely with the mononuclear variety.

In a given section areas may be seen where the walls are thickened and the alveoli are filled with exudate as described. Other areas will show no exudate (Fig. 5). In the less acute forms, organization is beginning to take place. Areas are seen where the exudate has disappeared from the alveoli and they have collapsed, permitting the thick walls to come in contact and making a strand of solid tissue with here and there the suggestion of an alveolar space. Other portions show fibroblastic invasion of alveolar exudate, the new cells originating in the walls (Figs. 5 and 6). Still others give the appearance of new connective tissue, with here and there a space either without endothelium or with a partial lining of cuboidal or columnar cells produced by the attempt at regeneration.

LABORATORY METHODS

A METHOD FOR THE DETERMINATION OF BLOOD VOLUME*

By Elizabeth Franke and Stanley R. Benedict, New York City

METHODS available for the determination of blood volume depend upon one of three general procedures. The direct method, devised by Haller, Wrisberg[1] and Welcker,[2] and modified by Plesch[3] and others[4] depends on direct estimation of the total hemoglobin content of the body. This procedure is probably the most accurate, but is applicable only where the life of the experimental animal is sacrificed, and the technic involved is laborious. The second group of methods, developed by Gréhand and Quinaud,[5] Haldane,[6] Van Slyke[7] and others,[8] depends upon inhalation of a gas (carbon monoxide) which will combine with a constituent of the blood (hemoglobin) so that by subsequent determination of the new compound found, or of the concentration of the gas in the blood, the blood volume may be calculated. This type of method has not met wide approval on account of the unsatisfactory technic involved, and because the results obtained show a wide variance from those given by other methods.

The third, and most widely used group of methods, depends upon the introduction of a known quantity of a substance into the circulating blood with subsequent determination of the concentration of the substance in a sample of blood withdrawn from the circulation. Such a procedure must depend upon the use of a substance for injection which remains practically unaltered in the blood stream for sufficient length of time to permit thorough mixing with the entire blood, and for withdrawal of the sample for analysis.

Acacia, employed by Meek and Gasser,[9] conforms to these conditions but the complicated analytical technic required to determine acacia with accuracy has limited the general usefulness of the method. An adaptation of this method, using the refractive index of the nonprotein fraction of the serum has been proposed by McQuarrie and Davis. This procedure is applicable to small quantities of blood and is probably quite accurate when a satisfactory refractometer is available, and conditions are properly controlled.

Theoretically, perhaps the simplest method of blood volume determination would depend on the introduction into the circulation of a nondiffusible nontoxic substance having an intense color, so that the concentration of the substance would be determined colorimetrically directly in a portion of plasma. Keith, Rowntree and Geraghty first proposed a method based upon this procedure and employed "vital red" a German dye, as the substance for injection. Vital red does not penetrate the corpuscles and leaves the circulation compara-

*From the Department of Chemistry, Cornell University Medical College, New York City, and the W. A. Clark Special Research Fund.

tively slowly. Difficulty in obtaining satisfactory samples of the dye in recent years has limited the availability of the method for general use. The work of Harris, Hooper, Smith, Belt and Whipple, and Dawson, Evans and Whipple has indicated that the colorimetric principle for blood volume determination as introduced by Rowntree and collaborators is the most generally satisfactory and simple procedure, provided one secures a dye which can be relied upon not to penetrate corpuscles or tissues within a reasonable period of time.

On account of the difficulties at present in obtaining reliable dye preparations which do not penetrate cells or tissues it occurred to us that hemoglobin might be used with advantage in the determination of blood volume. Hemoglobin is nontoxic, and owing to its high molecular weight and intense coloring power should be admirably adapted for use where a nondiffusible colored product is desired. Our experiments have shown that oxyhemoglobin does not penetrate the red cells to the slightest degree, and remains in the circulation without detectable alteration for periods of time quite sufficient to allow of thorough mixing with the blood and removal of samples for analysis. The final concentration of hemoglobin in the plasma is readily determined by comparison in a colorimeter with a portion of the solution injected, after suitable dilution. It is unnecessary to use purified hemoglobin for the determination. Results are exactly as satisfactory where a solution obtained by laking corpuscles with water, followed by addition of enough salt to make the whole solution isotonic, is employed. The hemoglobin employed need not come from the same species of animal as the one used in the experiment. We have used beef hemoglobin in our dog experiment with exactly the same results as where dog hemoglobin was employed. Injection can be made as rapidly as possible without detectable effect on the general condition of the animal.

DESCRIPTION OF THE METHOD

The principle of the method is the introduction into the blood stream of a solution of hemoglobin, prepared by laking blood, and the subsequent determination colorimetrically of the concentration of this hemoglobin in the plasma by comparison with a suitable standard mixture of hemoglobin and plasma. The volume of the corpuscles in the blood must also be determined. This can be done either with a hematocrit, or by the method described below, which depends upon addition of hemoglobin solution to whole blood, and the subsequent determination of the hemoglobin concentration in a portion of the plasma. The three main steps in the procedure are:

1. Withdrawal of a preliminary sample of blood to secure plasma for use in the standard solution, and to furnish a sample of blood for determination of the corpuscle volume.

2. Injection of hemoglobin solution.

3. Withdrawal of blood samples a few minutes later in which the concentration of hemoglobin in the plasma is determined colorimetrically.

PREPARATION OF HEMOGLOBIN SOLUTION FOR INJECTION

Centrifuge a known amount of blood (a few c.c. more than 0.5 c.c. for each kilogram of body weight of the subject) in a sterile centrifuge tube until

the corpuscles have settled fairly completely. Pipette off the plasma as completely as possible into a small graduated cylinder. Add to the cell residue in the centrifuge tube enough sterile water to make the total volume twice that of the original blood. Shake to thoroughly hemolyze the cells. Add 8.5 milligrams of NaCl for each c.c of water added, and mix by shaking. Centrifuge to remove white cells and debris, and pipette off the clear red solution. Approximately 1 c.c. of this solution is injected for each kilogram of body weight of the subject.

Technic.—Take a preliminary sample of blood in the usual way, using about 2 milligrams of potassium oxalate per c.c. to prevent clotting. This sample should have a volume of from 10 to 15 c.c. A portion of this blood (2 c.c.) is measured into a centrifuge tube for the corpuscle volume determination, or a small quantity is used for the hematocrit determination of corpuscle volume. The main portion of this blood is now centrifuged, and while this is going on the previously prepared hemoglobin solution (prepared as described above) is injected intravenously. The quantity used should be about 1 c.c. per kilogram of body weight, and must be accurately measured. Approximately 2 minutes after making the injection a sample of about 10 c.c. of blood should be withdrawn (either from an artery or vein) and if convenient a second sample should be withdrawn about two minutes later for a check determination. These samples of blood should be drawn into vessels containing 2 milligrams of oxalate for each c.c. of blood, and should be centrifuged as promptly as possible after thorough mixing with the oxalate.

PREPARATION OF THE STANDARD SOLUTION

The dilution of hemoglobin best adapted for colorimetric determination was found to be approximately that obtained by diluting one part of whole blood to two hundred parts with water. Since the color of plasma varies in different individuals, it is necessary to mix the standard with plasma in approximately the same quantity as is present in the unknown solution. Hence the standard solution is prepared by mixing proper proportions of the hemoglobin solution used for the injection with plasma obtained from the preliminary blood sample, and proper dilution of the mixture with normal salt solution. For preparing the standard solution, dilute one part of the hemoglobin used for injection to five volumes with 0.85 per cent salt, and one part of this solution again to ten volumes with the salt solution. Finally mix a portion of this solution with an equal volume of plasma obtained from the preliminary blood sample. This final solution is employed as standard solution in the colorimeter. For comparison, the hemoglobin-tinged plasma obtained by centrifuging the sample (or samples) withdrawn after the injection is diluted with an equal volume of salt solution. The standard is conveniently set at a height of 15 m.m.

CALCULATION OF RESULTS

Let S represent the height of the standard in millimeters, and Y represent the reading of the unknown in millimeters. Then $S/Y \times 2$ (since the plasma withdrawn was diluted 1:1 with saline) will represent the relative concentra-

tion of hemoglobin in the original plasma as compared with the standard, which figure we will term *C*. Then if *H* represents the number of c.c. of the hemoglobin solution injected, H/C × 100 will give the number of c.c. of plasma in the body. The number of c.c. of plasma in the body, divided by the percentage of plasma in the blood, and multiplied by 100 will give the number of c.c. of total blood in the body.

Example: Standard is set at 15 mm., unknown reads 18 mm. 15/18 × 2 gives 1.66. Then if 12 c.c. of the hemoglobin solution were injected 12/1.66 × 100 gives 734, the number of c.c. of plasma in the body. If the blood was 65 per cent plasma then 734/65 × 100 = 1129 c.c. of blood in the body.

DETERMINATION OF PLASMA PERCENTAGE IN BLOOD

Principle.—The method depends upon mixing hemoglobin with whole blood and determination of the concentration of hemoglobin in the plasma after centrifuging. It offers no special advantage over the use of the hematocrit but is quite accurate and may prove serviceable where a satisfactory hematocrit is not available.

Procedure.—To 2 c.c. of the preliminary sample of blood in a centrifuge tube add an equal volume of hemoglobin solution which represents a dilution of whole blood 1:10.13 (hemoglobin solution II). The mixture is centrifuged. One c.c. of the supernatant fluid is diluted to 10 c.c. with 0.85 per cent salt solution and this colored solution read in a colorimeter against a standard solution prepared by treatment of 0.5 of a portion of hemoglobin solution II

TABLE I

BLOOD VOLUME FIGURES FOR NORMAL DOGS

DOG	WEIGHT	PER CENT CELLS	PER CENT PLASMA	PLASMA VOLUME	BLOOD VOLUME	C.C. BLOOD PER 100 G. BODY WT.	BLOOD VOL. EXPRESSED IN FRACTION OF BODY WT.
20	13.46	32.0	68	725.3	1066.6	7.92	1/12.6
18	8.8	24.0	76	516.7	679.8	7.72	1/12.9
15	16.42	37.4	62.6	842.6	1346.1	8.19	1/12.19
14	10.68	37.0	63.0	530.0	841.3	7.87	1/12.6
22	11.3	37.4	62.6	700.08	1118.3	9.01	1/11.0
12	8.8	36.1	63.9	466.6	730.4	8.30	1/12.0
10	8.8	36.1	63.9	538.2	842.2	9.57	1/10.4
81	14.72	31.6	68.4	823.2	1205.6	8.10	1/12.2
81	14.80	41.3	58.7	676.8	1182.9	7.99	1/12.5
85	11.0	32.0	68.0	715.7	1078.5	9.80	1/10.19
85	10.86	28.0	72.0	744.4	1068.8	9.84	1/10.16
86	16.04	29.2	70.8	1133.3	1620.7	10.1	1/9.89
87	6.4	45.0	55.0	374.8	700.4	10.9	1/9.13
87	5.92	36.0	64.0	342.9	565.7	9.55	1/10.4
90	9.08	37.4	62.6	546.9	894.9	9.85	1/10.4
91	10.60	40.0	60.0	550.38	952.3	8.98	1/11.1
91	10.18	37.4	62.6	548.3	905.8	8.8	1/11.3
92	10.52	45.2	54.8	556.07	1059.7	10.0	1/9.9
93	15.94	40.0	60.0	653.96	1124.9	7.05	1/14.1

with an equal volume of clear plasma from the preliminary blood sample and dilution of the mixture to 10 c.c. with 0.85 per cent salt solution.

Calculation.—The figure obtained by dividing the reading of the standard by the reading of the unknown solution gives the relative concentration of hemoglobin in the solution. By dividing the total volume originally in the centrifuge tube by this figure we obtain the actual volume of total fluid present (plasma + hemoglobin solution added). By subtracting the volume of hemoglobin solution added we obtain the volume of plasma. Dividing this by the volume of whole blood and multiplying by 100 gives us the percentage of plasma in the blood.

Example.—Standard set at 15 mm.; unknown reads 12.2 mm. Then $15/12.2 = 1.22$. 4 c.c. (total volume in centrifuge tube) divided by 1.22 gives 3.278. Subtracting the 2 c.c. of hemoglobin solution added we get the figure 1.278 c.c. as the plasma present in 2 c.c. of blood. The $1.278/2 \times 100 = 63.9$ per cent of plasma in the blood.

CONTROLS OF THE METHOD

Hemolysis.—In this method, as in any which depends upon colorimetric determination directly upon the plasma, are disturbing factors. If care is exercised in drawing the blood, so that absolutely no clotting occurs, there will be little trouble from hemolysis. Very prompt centrifugation of the blood samples is also desirable, since blood is apt to hemolyze on standing, even for short periods.

In Table I the results are given of 19 experiments to determine the blood volume of dogs by the method described in this paper. In each case the hemoglobin solutions were injected into the femoral vein and the blood withdrawn through an oiled cannula inserted into the femoral artery on the same side.

TABLE II

VALUES FOUND IN REPEATED DETERMINATIONS

DOG	TIME INTERVAL	WT. IN KILOS	PER CENT PLASMA	PLASMA VOLUME	BLOOD VOLUME	C.C. BLOOD PER 100 G. BODY WT.	BLOOD VOL. EXPRESSED IN FRACTION OF BODY WT.
81		14.72	68.4	823.2	1205.6	8.10	1/12.2
	11 days	14.80	58.7	676.8	1182.9	7.99	1/12.5
85		11.0	68.0	715.7	1078.5	9.80	1/10.19
	11 days	10.86	72.0	744.4	1068.7	9.86	1/10.16
91		10.6	60.0	550.38	952.3	8.98	1/11.1
	7 days	10.18	62.6	548.3	905.8	8.8	1/11.2
87		6.4	55.0	374.8	700.4	10.9	1/9.13
	7 days	5.92	64.0	342.9	565.7	9.55	1/10.4

Local cocaine anesthesia was employed. It will be noted that the results vary from 7.7 c.c. to 10.9 c.c. per 100 grams of body weight, or from about 1/10 to 1/14 of the body weight. These results are in agreement with those reported by other observers with other methods.

Table II records duplicate determinations of the blood volume on four

dogs at intervals of seven or eleven days. The results show excellent agreement.

Studies were also made to determine the rate of disappearance of hemoglobin from the circulation. In these experiments samples of blood were taken from a cannula inserted into the femoral artery, at intervals of 1.5, 3, 5, 10 and (in one case) 20 minutes after the hemoglobin injection. The results are summarized in Table III. It will be seen that three minutes after the injection of the hemoglobin more than 98 per cent is still present in the blood stream. Indeed samples taken at 1.5 and at 3.0 minute intervals almost always show excellent agreement. At the end of five minutes the loss of hemoglobin averages about three per cent, and after ten minutes, 4.2 per cent.

Experiments have been carried out to test the accuracy of the method suggested for the determination of plasma and corpuscle volume both by comparison with hematocrit readings and by artificially increasing the corpuscle content of blood samples by removal of measured quantities of plasma. The maximal errors found by the latter method amounted to 2.6 per cent, so that we may conclude that the method is accurate to within two or three per cent.

Summary.—A simple method for the determination of plasma percentage and plasma volume in living animals is described. From these two factors the total blood volume can be calculated. The blood volume of normol dogs averages about 1/11 of the body weight. While the method has not yet been tried with other animals than dogs, there is no apparent reason that it cannot be applied to any species.

TABLE III

RELATIVE CONCENTRATION OF HEMOGLOBIN IN PLASMA AFTER ITS INJECTION

	(1.5 minute sample taken as 100)				
DOG	1.5 MIN.	3 MIN.	5 MIN.	10 MIN.	20 MIN.
81	100	96.2	96.2		
81	100	99.2		97.7	86.4
85	100	97.0	97.0	98.1	
85	100	95.9	94.9	93.0	
86	100		93.3	93.3	
87	100	95.5	95.0	90.0	
87	100	102.0	102.0	100.6	
90		100		96.9	
90	100	100.5	98.6	96.5	
91		100.	99.1	94	
91	100	101.0	99.7	98.5	
92	100		99.3		
93	100	96.0			

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[15]This principle was employed by Stewart, (Jour. Physiol., 1879, xxiv, 356) who added a solution of oxyhemoglobin to a known volume of centrifuged blood from which a known amount of plasma had been removed, and calculated the plasma volume from the dilution necessary to bring the standard solution to the same tint as that of the unknown (compared in hematinometers).
[16]This solution can be prepared by dilutions of a portion of hemoglobin solution used for injection with four volumes of 0.85 per cent salt solution, or whole blood may be diluted to ten times its volume with distilled water and the resulting solution treated with 85 milligrams of salt for each c.c. of water added.

A STANDARD METHOD FOR PREPARING AND STANDARDIZING LIPOIDAL ANTIGENS FOR THE WASSERMANN TEST*

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SINCE the time when the complement-fixation test has found its practical application as the Wassermann reaction in the diagnosis of syphilis, the study of the nature of lipoidal antigens has deservedly attracted much attention.

Wassermann and Bruck in their original investigations used first watery and later alcoholic extractives of syphilitic livers with the idea that they were dealing with a specific complement-fixation test. This idea, however, was soon abandoned when the nonspecific nature of the syphilitic antigens was known from actual tests conducted with alcoholic extractives of normal tissue rich in lipins. (Marie and Levaditi, Landsteiner, Porges, and Meier Weil and Braun, Noguchi, etc.)

On further study of such tissue extractives (Sachs, Browning and Cruikshank, Walker and Swift) introduced the addition of very small amounts of cholesterin, thus increasing considerably the sensitiveness of plain extracts. Noguchi observed that cholesterin, as well as certain other undesirable products of the nature of proteins soluble in alcohol (that are usually found in ordinary tissue extractives), rendered the antigens less specific in direct proportion to their quantity. Consequently Noguchi devised a method by which he could separate the relatively pure lipins from the other undesirable extractives. In general his method consists in precipitating lipoids from an etherial solution by the addition of acetone.

As a natural outcome of the above studies we now possess four different

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antigens. The syphilitic tissue extractive, the normal tissue extractive, the cholesterinized or reenforced tissue extractive, and the acetone-insoluble fraction of Noguchi.

A casual examination of the literature on the Wassermann reaction will disclose the fact that the study of the antigen (the most important of all items entering into the reaction) has so far been rather neglected. Much time and labor have been spent in finding some new method to produce a more potent or sensitive antigen, but relatively little has been done to establish a strictly scientific method for the standardization of the complement-fixing value of Wassermann antigens. Thus, for example, all the pioneers in this field of research, Wassermann, Bruck, Sachs, Browning and McKenzie, Thomas and Ivy, Walker and Swift, Field, M. Stern, etc., still use either an arbitrarily fixed dose of some preparation, or a known fraction of the dose which by itself is not anticomplementary. There is no doubt that almost all of them make a preliminary titration of the relative fixing value of their antigen to determine the specific and nonspecific (anticomplementary) limits; but the way such titrations are made is invariably open to criticism of being far from scientific.

To illustrate the above statement we will briefly explain the method of titration used by the New York Department of Health Laboratories. They take for example as their dose a fixed amount of a given strength of dilution, one-fourth of which should give complete fixation of the unit complement used in the actual test, in presence of a strongly positive serum and which in quantities 3 to 4 times as large should not be anticomplementary.

Before I proceed any further, let me state in advance that given an antigen, irrespective of the method of preparation, the degree of specificity will bear a constant ratio to the amount of lipins present, provided the nonlipin fraction in it is always constant.

This fact, long recognized by workers in this field, such as Field, has led to the attempt of standardizing antigens through determining the amount of solids found in a definite volume of the given antigen. Unfortunately the relative proportion of the lipin and nonlipin fraction in an antigen is not always the same. Field[1] uses an alcoholic extract of guinea pig hearts reenforced with cholesterin to half saturation. The total solids in his antigens vary from 5 to 8 milligrams per c.c. This variable item is corrected in the final dilution of the antigen, so that one c.c. of this dilution will always represent 0.1 mg. of solids. Noguchi, on the other hand, goes a step further in getting rid of the nonlipin fraction by precipitating it with acetone, thus obtaining the relatively pure semisolid lipins in a weighable form. This method would be the most promising of all the rest were it not for the fact that the acetone-insoluble fraction of tissue extractives does not represent their total antigenic value. On running parallel tests with both fractions, acetone-insoluble and acetone-soluble one can easily demonstrate the fact that acetone-soluble fraction retains a great part of the antigenic element, though it is less specific. It is partly due to this fact that as antigens, comparatively higher concentrations have been used of the acetone-insoluble fraction.

The following will be an attempt to handle the question of antigens in mathematical terms. The basis of all complement-fixation reactions is the degree of complement deviation in terms of hemolysis (the indicator). Consequently the standardization of the hemolytic system precedes that of the antigen. In spite of so many variations introduced in the original Wassermann technic, the standard unit of 1 c.c. of 5 per cent suspension of sheep cells has been more or less adhered to.

Wassermann in his original work used whole blood to make the 5 per cent suspension. This was later replaced by washed, packed cells, thus eliminating another source of error, *i. e.* the variability of the corpuscular volume in whole blood.

Disregarding the possibility of nonuniformity in the resistance of cells of different sheep, let us temporarily adopt a standard unit of cells to which our antigen standardization has to be adjusted. The original unit of 1 c.c. of 5 per cent suspension is inapplicable as a standard unit for two reasons: It demands unnecessary waste of material, especially the complement, and what is far more important, its undue bulk renders it less sensitive as an indicator.

During the last decade the general tendency among workers in this field has been to reduce the Wassermann unit into ½, ¼, or even ⅕ of the original. To safeguard against all these variations let us adopt as our unit 1 c.c. of 1 per cent suspension, as this amount, in addition to increased sensitiveness and economy in material, has the advantage of being easily convertible into the original 5 per cent or any of its fractional units.

Then our unit complement would be the least amount that is capable of hemolyzing completely 1 *c.c. of* 1 *per cent suspension of properly sensitized cells.*

There is much divergence of opinion regarding the proper sensitization of cells. From 2 up to 10 units of sensitizer have been used by different authors. Fortunately in our antigen titrations we depend on the combined action of complement and sensitized cells. In this way any excess or deficiency in any single item is finally corrected by the other in the titration of the complement prior to antigen titrations.

In our titrations we have invariably adopted the method of titrating the complement against 2 units of sensitizer, which we found very satisfactory. The antigen unit would then be *the least amount capable of deviating completely one unit of complement in presence of exactly one unit of specific reagen.* The significance of this has been only casually dwelt upon. The present system of pluses is a qualitative denotation after all. No two 4-plus sera have necessarily the same amount of specific reagen. Therefore, any attempt to fix a standard for the antigen without at the same time determining the unit specific value of the serum against which the antigen in question is to be titrated is bound to fail. A standard unit of specific reagen, then, *is the least amount capable of deviating one unit of complement in the presence of one unit of antigen.* Having thus defined our terms, we now proceed to the actual application of our method.

TECHNIC OF THE DETERMINATION OF THE STANDARD UNIT OF ANTIGEN

The whole process is divided into three steps: (1) the determination of the minimal fixing value in presence of a known strong positive serum. (2) the determination of the approximate standard unit of the antigen. (3) the determination of the exact antigenic and specific units.

The process of determining the minimal fixing value of the antigen does not differ much from the technic actually carried in any other laboratory: Make an arbitrary dilution of the antigen, arranged in a series of 5 to 10 test-tubes. Each tube gets one unit of complement.

The unit of the complement is determined in the usual way by finding the minimal amount capable of hemolyzing completely 1 c.c. of 1 per cent sensitized sheep cells. The preparation of 1 per cent sheep erythrocytes needs no special comment, as long as it is serum-free and actually measured. As far as the sensitizer is concerned, two units of sensitizer per unit of cells is safe enough, although a half and half proportion would be more scientific.

Then we add an arbitrary amount of known strong positive serum, say 0.1 c.c. and finally the antigen is added in gradually decreasing doses, say from 1 c.c. to 0.05 c.c. All tubes are now incubated at 37° C. for 30 minutes and 1 c.c. of 1 per cent sensitized sheep cells are added into each tube, incubated another 30 to 45 minutes and the final readings taken. The smallest amount of antigen that gives complete fixation of one unit of complement as used in the tests is considered the minimal fixing value of the antigen in question.

It is customary in different serologic laboratories to use an excess of complement, from 2 to 5 complement units. We adopted the use of 1 such unit for the reason that by doing so, besides the economy in material, we also eliminated a variable factor, and our results can easily be converted at any time in terms of any other laboratory findings.

Having thus determined the minimal fixing value of an antigen, we now proceed to determine the approximate standard unit antigenic value. Suppose the minimal fixing value of the antigen to be 0.5 c.c. of 1:100 dilution. We now make 1:50 dilution (twice as concentrated), and arrange 6 rows of 6 tubes each. (More tubes can later be added if necessary.) Every tube gets the same amount (0.1 c.c.) of the known strong positive syphilitic serum. Next, the complement is added in regularly increasing units, so that the first tube of each row gets 1 unit, the second 2 units, etc., the last tube of each row getting 10 units. Finally we add the antigen in regularly decreasing doses so that all tubes of the first row get 0.05 c.c. each, those of the second row 0.10 c.c. each, etc., tubes of the last row getting 0.50 c.c. each. The deficiency in total volume is made up by adding saline. Then tubes are shaken and incubated for 30 minutes at 37° C., when one unit of sensitized cells is added to each tube. Again they are shaken and further incubated for 30 to 45 minutes at the same temperature, whereupon readings are taken and results tabulated. Table number I will make this clear.

TABLE I

ANTIGEN	COMPLEMENT IN UNITS					
IN C.C.	1	2	3	4	5	10
0.05	c	c	c	c	c	c
0.10	p	c	c	c	c	c
0.20	n	p	c	c	c	c
0.30	n	n	p	c	c	c
0.40	n	n	n	p	c	c
0.50	n	n	n	n	p	c

Explanatory note: c denotes complete hemolysis, p varying degrees of partial hemolysis, and n no hemolysis at all.

CONCLUSIONS

Table I shows that the approximate standard unit of the antigen in question lies between 0.10 and 0.20 c.c. of the 1 to 50 dilution, probably at 0.15 c.c. But we do not know yet to what degree the antigen unit thus determined is influenced by the strength of the specific reagen. The table also gives an approximate estimation of the extent of this influence, because we at least know that this value is not below 5 units in the standard quantity of 0.10 c.c. above used, since by our definition, 0.10 c.c. of the serum is capable of fixing at least 4 units of complement. Its failure to fix more than 4 units of complement may be due to two reasons; either the serum has no more than 4 units of specific reagen, or if it has the insufficiency of the antigen added renders the serum ineffective as far as fixation is concerned. This aspect of the question will be taken up in the final stage, where the exact unit values of antigen and reagen are definitely fixed. Here we use only one unit of complement for all tubes, varying our antigen and specific reagen within their approximate unit limits.

TABLE II

SERUM	ANTIGEN 1:50 DIL.			
IN C.C.	0.05	0.10	0.15	0.20
0.005	p	p	p	p
0.010	p	p	p	n
0.015	p	p	n	n
0.020	p	p	n	n

Explanatory note: p denotes varying degrees of partial hemolysis, n denotes complete inhibition of hemolysis.

FINAL CONCLUSION

Table number II shows that 0.005 c.c. of the serum fails to exhibit a unit of reagen, while 0.01 c.c. of serum with 0.20 c.c. of antigen fulfill the established requirements for a unit. 0.015 c.c. of serum with 0.15 c.c. of antigen also fulfill the requirements. Either one of the combinations might be safely used as a standard unit. In our experience we always found it safer to favor the antigen at the expense of the serum, consequently we would adopt as standard unit 0.01 c.c. for the serum and 0.20 c.c. for the antigen in a dilution of 1:50.

The fallacy of standardizing the antigen against any given strong positive serum lies in the fact that all positive sera are not quantitatively alike.

Every laboratory, through habit or for certain special reasons, has sanctioned a definite dose for the specific serum. This dose varies from one drop to 0.20 c.c. The other ingredients vary more or less in proportion to the serum used. If now a serum in the fixed dose of say 0.10 c.c. results in complete fixation of the complement used, it is called a 4-plus serum. By further reducing the amount of serum a limit soon is reached, where any further reduction of the amount will fail to fix the complement completely. Supposing this lowest quantity to be 0.01 c.c., the original dose of 0.10 c.c. must then contain 10 absolute units of specific reagen. By standardizing an antigen against such a strong positive serum we ignore the law of multiple proportions as exhibited by the classic studies of the Bordet and Ehrlich schools; (V. Dungern,[2] Morgenroth and Sachs,[3] Manwaring,[4] etc.).*

Assuming that we have made clear the question of an absolute unit-fixing value of the antigen we now proceed to determine its specific (non-anticomplementary) and hemolytic values before we decide about its adaptability in the Wassermann reaction. These two values are arrived at as usual by preparing comparatively more concentrated dilutions of the antigen, so that the total volume used in the previous tests is not exceeded.

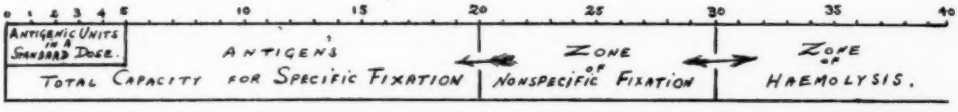
It is more or less generally agreed upon that with a good Wassermann antigen the minimal anticomplementary value should at least exceed 4 or 5 times the minimal fixing value, and that the more the anticomplementary value exceeds the specific unit, the better and safer the antigen is considered.

The hemolytic element in the antigen, is not often met with. However, occasionally a tissue extractive will exhibit hemolytic properties in amounts very dangerously small, thus interfering with its specific values. Such extractives should be discarded as useless. A hypothetical antigen answering the above description can be represented graphically as follows:

TABLE III

A GRAPHIC REPRESENTATION OF THE RELATIONS EXISTING BETWEEN THE VARIOUS PROPERTIES OF AN IDEAL ANTIGEN

IN TERMS OF COMPLEMENT UNITS


0 1 2 3 4 5 10 15 20 25 30 35 40
Antigenic Units in a Standard Dose.
Antigens
Zone of
Zone of
Total Capacity for Specific Fixation
Nonspecific Fixation
Haemolysis.

We, therefore, collected antigens from various laboratories in New York City and determined carefully, in accordance with the scheme already outlined, the specific and nonspecific antigenic units. We then computed the ratios of these two values, which for the sake of convenience we called the Specific Coefficient. For example, supposing the unit antigenic value of the given dilution to be 0.10 c.c. and the beginning anticomplementary value of the same dilution at 0.40 c.c., the specific coefficient then would be 0.10/0.40

*If we could only abolish the unscientific method of pluses and record our results in terms of the lowest complement-fixing values of the specific serum, as it is already done with spinal fluids in most laboratories, we would establish a relatively accurate quantitative method, influenced by only one variable item, the antigen, whose scientific standardization we have undertaken to discuss in the present article.

= ¼, which means that the antigenic unit is ¼ of the beginning anticomplementary unit.

The antigens under investigation were then grouped, according to their specific coefficients under the following classes: In all 15 antigens were thus examined:

Class 1. All antigens with Specific Coefficient of 1 or above 1. Such antigens are very unsafe, and should be discarded. Out of 15 antigens 2 came under this class, one plain alcoholic and one cholesterinized.

Class 2. Antigens with a Specific Coefficient of ½. Of these we had four, two plain alcoholic, one cholesterinized, and one acetone-insoluble fraction.

Class 3. Antigens with a Specific Coefficient ¼. Two alcoholic and two cholesterinized antigens.

Class 4. Antigens with a Specific Coefficient of ⅕. Under this class we had four; one plain alcoholic, one acetone-insoluble fraction, and two cholesterinized.

Class 5. Antigens with a Specific Coefficient of 1/20. Of this class we had only one, a cholesterinized antigen.

Excepting one cholesterinized antigen, with a specific coefficient of 1/20, the Specific Coefficient of all the rest varied between ½ and ⅕; in other words, the total fixing capacity of these antigens did not exceed 5 units.

Noguchi, in his book on Serum Diagnosis of Syphilis, puts down the total specific fixing value of his antigen as 5 units. Kolmer, referring to the Noguchi antigen, makes the statement that it contains a fixing capacity of 10 to 20 units. Thiele and Emleton[5] use 0.20 c.c. of 1:10 dilution of acetone-insoluble lipins, which they state to be from 50 to 100 units in complement-fixing value. Bronfenbrenner,[6] in his article "A Procedure for the Serum Diagnosis of Syphilis", says, "It is possible to prepare this antigen (acetone insoluble fraction) in such a way that 1/10 of the anti-complementary dose of it contains from 10 to 100 antigenic units, depending upon the serum against which it is tested."

As far as Noguchi's own statement is concerned, it conforms with ours. Kolmer's figures also must undoubtedly be correct, the higher values he gets being due in all probability to the strong positive sera against which his antigens are titrated. Otherwise, as we have already stated above, were the antigens in question titrated against a single, or at the most, a double unit of specific reagen, the results would not have been very different. Coming to Bronfenbrenner's statement, granted that the samples of acetone-insoluble fractions in our possession were inferior to his, we still fail to understand the wide limit of variation, even assuming that he standardized his antigens against varying degrees of positive sera, considering that an antigen has been used, whose mode of preparation is more standard than of any other Wassermann antigen. Further it must be clearly understood that the unit value of 10 to 100 is contained as stated by Bronfenbrenner, in 1/10 the anticomplementary dose, consequently, the total fixing value would be from 100 to 1000 units.

Although Bronfenbrenner does not state the method by which he determines the specific fixing capacity of his antigen, his statement that the

antigenic value depends upon the serum against which it is tested, leads us to infer that he arrived at this conclusion by making quantitative Wassermann tests on different grades of positive sera. According to the Noguchi method of determining quantitatively the specific reagen, the standard amount of patient's serum is reduced to the lowest possible limit that is capable of still fixing one unit of complement. For example if a serum fixes one unit of complement in $\frac{1}{50}$ or $\frac{1}{100}$ the standard dose ordinarily used for qualitative determinations it is considered as 50 or 100 unit serum. Thus it is self-evident that, although the quantitative fixing value of the serum used is 50 or 100, the antigen, in the process of determining this value is titrated against a fraction of this value and only *one unit of complement.*

The discussion of the subject of specific coefficient would be incomplete without bringing forth a very important fact concerning the relationship of the specific coefficient with the actual specificity of the antigens under consideration. It must be clearly borne in mind that the specific coefficient gives the relative, not the absolute specific value of an antigen. An antigen with a relatively high specific coefficient, say $\frac{1}{5}$, might fail to detect a weak positive serum which another one with a lower specific coefficient, $\frac{1}{2}$, might detect. After all, the specific coefficients were determined on very strong positive sera that could be detected by any antigen conforming with the ordinary antigenic requirements, while most of the discrepancies in Wassermann reactions arise from doubtful or weak positive sera.

We dealt with this very interesting question by running several hundred routine Wassermann tests, simultaneously with all antigens in our possession.* The results are the following:

Cholesterinized antigens give more positives than crude extracts do, with the difference that not all positives with cholesterin antigens are syphilitic. The crude alcoholic extracts detect from 40 to 60 per cent of the sera that are positive with the cholesterin antigens, with the difference, however, that false positives are rare exceptions. Our figures with plain alcoholic antigens are lower than those given by other investigators, especially when they used the ice-box fixation method.

The degree of absolute specificity bears a distinct relation to the specific coefficient. This, however, is not an unexpected finding when we consider the significance of the specific coefficient. An antigen with a total fixing value of 20 units should be superior to one with only 4 units, as the former can be used in amounts as small as $\frac{1}{10}$ the anticomplementary dose and still represent 2 units while the latter would represent the same value in $\frac{1}{2}$ the dose, thus rendering its use rather unsafe.

On further investigation we found that the amount of solids contained in a definite volume of antigen, excluding cholesterin and alcohol-soluble

*Our Wassermann technic consists in using inactivated serum in amounts of 0.05 c.c. for cholesterinized and 0.05 and 0.10 c.c. for plain alcoholic antigens. The complement, guinea pig's serum, is titrated daily against 2 to 4 units of antisheep sensitizer, 0.50 c.c. of 1 per cent sheep cells being used as indicator. For the actual tests 2 such complement units are used. Of cholesterin antigens 2 units and of plain alcoholic extracts 2 to 4 units are used. The antigenic unit determinations were made as described. The total volume in each tube is always 1 c.c. A primary incubation of 30 to 45 minutes is done in a water-bath at body temperature for the fixation of the complement. At the end of this time 0.50 c.c. of 1 per cent sensitized sheep cells are added. In about 30 to 45 minutes, when the antigen, complement, and serum controls show 100 per cent hemolysis, the readings are taken.

proteins, bears a direct ratio to the degree of specificity of the antigen in question. The major part of these solids is made up of lipins and certain other bodies akin to it, the discussion of which we will take up later.

The action of cholesterin in Wassermann antigens is still a debated question. There is no doubt that cholesterin increases considerably the sensitiveness of plain extractives, but this is always at the expense of their specificity. In trained hands it is a valuable aid to detect weak positive sera, especially in cases under treatment; but its results must always be checked up with a reliable plain extractive. On the other hand cholesterinized antigens in our experience were almost worthless where active sera were used.

The statement that alcohol is a general protein coagulant is of relative truth only, depending primarily on the relative purity of the alcohol used. For by extracting dry muscle tissue with varying grades of alcohol, from 50 per cent to absolute, we noticed that the more dilute the alcohol, the more protein it will hold in solution. Again the more concentrated it is, the richer it will be in nonprotein extractives.

By far the most interesting of these findings was the peculiar relationship between an antigen's total of solid contents and its specificity. Table Number IV shows very clearly all these relations in the case of a few antigens, taken at random and showing all extreme possibilities.

TABLE IV

THE SPECIFIC COEFFICIENTS OF VARIOUS ANTIGENS IN RELATION TO THE TOTAL SOLID CONTENTS OF SAME

KINDS OF ANTIGENS USED		TOTAL SOLIDS IN GMS. PER C.C.	ANTIGENIC UNITS IN C.C.	MINIMAL ANTI-COMPLIMENTARY VALUES IN C.C.	SPECIFIC COEFFICIENT	STAND. ANTI-GENIC DOSE IN C.C.	ANTIGENIC UNITS IN STANDARD DOSE
No. 1	(Plain alcoholic extr.)	0.002	0.50	1.00	$\frac{1}{2}$	0.10	0.20
No. 2	(Cholesterinized No. 1)	0.006	0.10	0.25	$\frac{1}{2.5}$	0.10	1.00
No. 3	(Plain alcoholic extr.)	0.012	0.50	1.00	$\frac{1}{2}$	0.50	1.00
No. 4	(Cholesterinized No. 3)	0.016	0.025	0.50	$\frac{1}{20}$	0.10	4.00
No. 5	(Aceton. Insol. Lipins.)	0.030	0.10	0.20	$\frac{1}{2}$	0.10	1.00
No. 6	(Noguchi Antigen)	0.030	0.04	0.20	$\frac{1}{5}$	1.10	2.50
No. 7	(Plain alcoholic extr.)	0.026	0.005	1.00	$\frac{1}{200}$	0.25	50.00
No. 8	(Cholesterinized No. 7)	0.030	0.05	2.50	$\frac{1}{50}$	0.50	10.00

Explanatory notes: Antigen No. 5 is prepared in accordance with the directions of Noguchi: 0.30 gms. of the lipin mass plus 1 c.c. of ether, plus 9 c.c. of methyl alcohol, thus representing 0.03 gms. of solids per 1 c.c. The figures given are from values obtained by titrating it against inactivated specific sera.

Figures in antigen No. 6 are taken direct from Noguchi's titrations as given in his book.

Antigen No. 7 was prepared in accordance with the technic described in the latter part of the present article.

Antigen No. 8 is the cholesterinized form of antigen No. 7. While the former shows 50 units per standard dose, it detects only 50 to 60 per cent of those sera that are positive with antigen No. 8.

These findings led us to attempt to prepare an antigen free, as far as possible, of all elements of error and specific in action. On account of the peculiar relationship between an antigen's total of solid contents and its relative specificity, and given that the highest yield in solids is obtained by

keeping the strength of alcohol as high as possible, we devised a technic, whereby it was possible to keep the alcohol at the original strength throughout the whole process of extraction. Thus we were not only eliminating the possibility of diluting the original strength of the alcohol used; but also avoiding the presence of those undesirable water-soluble by-products, which are protein in nature. Noguchi calls them collectively the "proteotropic substances" as they fix complement when mixed with certain proteins such as nucleoproteins, peptone, albumoses and other autolytic decomposition products of protein.

TECHNIC OF PREPARING ANTIGEN

Heart muscle tissue (ox, guinea pig, or human) is carefully freed of its connective tissue and fat, washed thoroughly under running water for an hour and gently squeezed from time to time to free it of blood. It is then wiped in cheese cloth, cut into small cubes and chopped finely in a meat chopping machine. Both the liquid and the solid parts are collected and the latter transferred into a meat pressing machine. The liquid that drains out is collected into an evaporating glass jar to which the fluid portion collected during the chopping process is added and there evaporated at body temperature or under the sun to a dry powder. Likewise the solid parts as they come out of the pressing machine are made into thin, flat cakes and dried immediately. The time for drying should not exceed a few hours as otherwise protein disintegration due to autolytic and saprophytic changes might vitiate the results.

The dry tissue is now passed through a nut-grinding machine and then pulverized in a mortar. To this the powder residue of the liquid portion is also added.

To 10 parts of the powder by weight 90 parts of absolute ethyl alcohol of the purest quality obtainable are added, then shaken well and put away in the incubator for extraction. The bottle is shaken once or twice daily. At the end of the week the supernatant amber-yellow clear fluid is decanted carefully into a tightly stoppered glass bottle and stored away in the ice box.

An antigen prepared in this way is very rich in extractives. The chemical composition of these extractives is very complex. The major part is made up of various aminophosphatids. The rest consists of fats, fatty acids, especially stearic and just a faint trace of cholesterin and soluble proteins.

In the course of a few months, during the time the antigen is kept in cold storage, certain chemical changes take place. The fat and fatty acid fractions combine with the alkaline elements present in the extract, thus forming neutral salts, soaps, which being insoluble in alcohol, are precipitated in the form of white chalky granular masses. Part of the fats is also precipitated as such, being caught among the granules of the soaps. Thus the antigen gets seasoned automatically.

An example of such an antigen is antigen No. 7 of the last table. Compared with the other antigens in the same table it shows 0.026 gms. of solid per 1 c.c. which was exceeded in this respect only by the Noguchi antigen which is but artificially made to contain 0.030 grams per 1 c.c.

Its specific coefficient is from 10 to 100 times less than that of any other antigen examined. The number of units per standard amount as actually used in the Wassermann test is at least 10 to 15 times greater than that of the best antigen prepared by the wet method.

As far as its absolute specificity is concerned, this could be ascertained only by performing actual Wassermann tests on a good number of sera, using antigens prepared by both methods, moist and dry. This was done on approximately one thousand cases taken at random, the results being the following:

1. Plain extracts of the dry method compared with plain extracts of the wet method give from 3 to 5 per cent less positive fixations, with the difference, however, that such undetected positives come under the class of weak or doubtful fixations. In the majority of these cases the history and signs are against syphilis. In other words, reactions with the dry method extract are either clear cut positives or negatives with a very small percentage of doubtfuls, and nonspecific fixations are extremely rare occurrences.

2. Cholesterinized extracts of both methods, when compared together are subject to the same remarks as stated under plain extracts.

3. Plain extracts of the dry process compared with cholesterinized antigens of the wet process give results which are indicated in the following table, No. V. The percentages given are computed on 350 undoubtedly syphilitic cases under constant observation.

TABLE V

TABLE SHOWING THE RELATIVE FIXING VALUES OF PLAIN DRY TISSUE EXTRACTIVES AND CHOLESTERINIZED WET TISSUE EXTRACTIVES

OF 350 UNDOUBTEDLY SYPHILITIC CASES, BY HISTORY AND SIGNS

ANTIG. NO. 7 PLAIN DRY DETECTS AS	CHOLESTERINIZED ANTIGEN OF WET METHOD DETECTS AS: Strong Positive	Weak Positive	Doubtful	Negative	Total for Antigen No. 7. Plain Dry	Difference between Antigens
Strong Positives	42 %	4 %	0 %	0 %	46 %	– 1.5 %
Weak "	2.5 %	8 %	7.5 %	0.5 %	18.5 %	– 5 %
Doubtfuls	0.5 %	5 %	7.5 %	2.5 %	15.5 %	–10.5 %
Negatives	2.5 %	6.5 %	11 %	0 %	20 %	+17 %
Totals for Cholesterinized Antigen	47.5 %	23.5 %	26. %	3. %	100. %	

Table V shows that strong and weak fixations are almost equally well detected by both antigens. The main discrepancy lies in doubtful and negative fixations. Cholesterinized antigen gave 26 per cent doubtful fixations and only 3 per cent negatives out of 350 undoubtedly luetic cases, while plain extractives of dry method gave 20 per cent negatives with 15.5 per cent doubtfuls.

The total difference of 17 per cent in favor of cholesterinized antigen is, however, of minor significance; because the great majority of cases that gave doubtful and negative fixations were under prolonged specific treatment. Furthermore, weak fixations with cholesterinized antigens should be taken

very reservedly. Chronic cardiacs, nephritics, certain acute infectious diseases as: scarlet fever, measles, pneumonia, septicemias; may at times give rise to nonspecific fixations with cholesterinized antigens.

To sum up, we can state with full confidence that plain alcoholic heart extractives, guinea pig or human, prepared and standardized as outlined in the present article, are preferable and less liable to give misleading results than cholesterinized antigens such as in general use for the performance of the Wassermann reaction.

I wish to thank Dr. Max Kahn, the Director of Laboratories, for his kind interest in this work.

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A SIMPLE DEVICE FOR THE DEMONSTRATION OF HEART BLOCK IN THE STUDENT LABORATORY*

By Nathan B. Eddy, M.D., Edmonton, Canada

THE methods in common use in the student laboratory for the production of heart block are the Gaskell clamp, ligature of the auriculo-ventricular junction and section of the tissue between auricle and ventricle. The Gaskell clamp in the hands of students is awkward to manipulate. It is heavy and must be balanced nicely to keep it in position without pressing unduly upon the auricle. It is an additional piece of apparatus to be kept clean and in working order. It has the very decided advantage of producing little or no injury so that it can be loosened and reapplied if the first attempt is unsuccessful. Ligature of the auriculo-ventricular junction is simple but one must exercise great care to tighten the ligature without jiggling the heart lever, distorting the record. It is difficult to grade the amount of constriction secured with the ligature and attempts to loosen it are very apt to damage the heart. On this account, failure at the first attempt in many cases necessitates the making of a new preparation. Section of the tissue uniting auricle and ventricle is unsatisfactory in that it requires too fine a nicety of technic to show any gradation between normal beat and complete heart block. Obviously the damage done is irreparable.

The apparatus herein described developed in the student laboratory. In principle it was first tried by one of our students, Mr. F. E. Wait, on his own initiative. It requires no special apparatus since the parts of which it is composed are usually available in a teaching laboratory. It requires no skill

*From the Physiological Laboratory of the University of Alberta, Edmonton, Canada.

for its successful application and a minimum of care to avoid distortion of the record. The damage done by it to the conducting pathway is slight, if any, allowing repeated trials to be made with the same preparation. It is not entirely rigid so that the auricular beat may show even when the constriction is tight.

The parts of the apparatus assembled are shown in Fig. 1. The essential part is a piece of silver wire (wire gauge number 26) doubled and run through a piece of fine glass tubing (inside diameter 2 mm.). The wire projects in a loop from one end of the tubing, making a "snare" for the heart. The ends of the wire projecting from the other end of the tubing are caught in

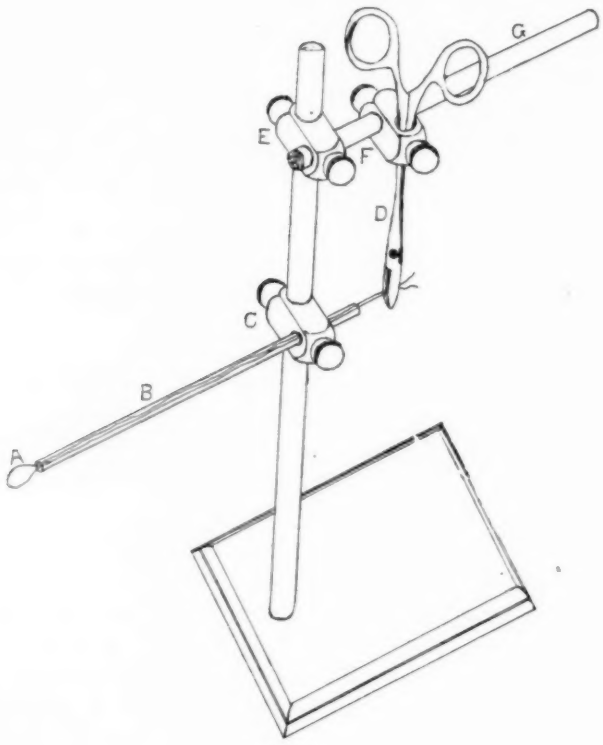

Fig. 1.

a hemostat. The heart is drawn up through the wire loop as far as the auriculo-ventricular groove and then is connected to the heart lever. The glass tubing is supported in a clamp on a stative with its smoothed end in contact with the heart. The "snare" is tightened about the heart by simply drawing the wire through the tube, slowly and steadily, as desired. The rod *G* and clamp *F* (Fig. 1) are not essential, but serve to support and steady the hemostat. The movement of the clamp and, therefore, of the hemostat is made smooth by applying a drop of oil to the rod. The clamp also makes it possible to arrest and hold the constriction attained at any time.

Tracings made with this device are shown in Figs. 2, 3, and 4. The tracing in Fig. 3 is especially of note as it is a part of a series of seven "heart

Simple Tachycard
3-1 Block
2-1 Block
Normal
Simple Tachycard
3-1 Block
Simple Tachycard

Fig. 2.

Sinus Tightens
Sinus loosens
Sinus Tightens
Sinus loosens

Fig. 3.

Sinus Tightens
Complete block
Ventricle beating independently
Sinus loosens

Fig. 4.

blocks'' obtained in one preparation in rapid succession with complete recovery of normal sequence of cardiac activity intervening each time. Both records in Fig. 2 were made from the same heart.

This apparatus for student's use was devised for and is particularly applicable to the frog's heart. It might be possible to apply it to the turtle's heart by using heavier wire with more spring and larger glass tubing. The Gaskell clamp, however, works entirely satisfactorily on the heart of the turtle since it is easy to make simultaneous records from auricle and ventricle.

THE EXPOSURE OF THE CILIARY GANGLION IN THE DOG FOR USE IN EXPERIMENTAL WORK*

By A. R. Cooper and J. T. Groot, Chicago, Ill.

THE literature bearing upon the pharmacology and physiology of the ciliary ganglion is lacking in clarity and precision, especially concerning the operative procedures involved in the exposure of that organ (cf. McGuigan: Jour. Lab. & Clin. Med., 1920, 6 (3): 161). Therefore at the suggestion of Dr. McGuigan we have developed a method which was demonstrated at the joint session of the Pharmacological and Physiological Societies at the meetings of the American Association for the Advancement of Science held December, 1920, at the University of Chicago. And since numerous requests for information as to the exact technic used have been received we thought it advisable to present the method in detail with the hope that it might be of benefit to others.

METHOD

The animal is anesthetized in the usual manner and then fastened upon the table on its side. It is then tracheotomized and the anesthetic administered by means of the ordinary ether-bottle. After this has been done, the common carotid artery on the side to be used is ligated in order to lessen subsequent hemorrhage, particularly in the orbit. Beginning at a point, one to two centimeters medial to the medial commissure of the eye, a curved incision is made through the scalp and extending back over the head close to the median line to the base of the ear. From the anterior end of this incision another is made at right angles to it, extending to the corner of the eye, around the rim of the upper eyelid to the zygomatic arch and thence for a centimeter or two farther downward. The flap thus marked out is reflected laterally, care being taken to secure the superficial temporal vessels situated just in front of the base of the ear. The temporal muscle thus exposed is carefully raised from the skull, beginning at the center of its upper margin and working downward in both directions, proceeding cautiously in the region of the orbit. Replace the muscle temporarily and then remove the zygomatic

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arch. This is done by dissecting along its upper and lower borders, sawing through its roots as far backward and forward as possible, the anterior cut coming well beneath the eyeball, and then raising it from the underlying tissues which obviously necessitates cutting through the lower end of the orbital ligament. The temporal muscle can now be easily dissected free from the coronary process of the mandible. The coronary process is then removed with bone forceps, or the jaw depressed, by stuffing a towel into the animal's mouth. Next the orbital ligament is cut away with scissors, injury to the insertions of the extraocular muscles which lie immediately beneath it being avoided.

A superficial pad of fat covering these muscles will be encountered; it is carefully dissected away with forceps, beginning well forward and working backward. Locate the insertion of the external rectus muscle; cut through it close to the sclera; holding the free end with a hemostat, carefully dissect it back towards its origin in the depths of the orbit, and sever it with a pair of

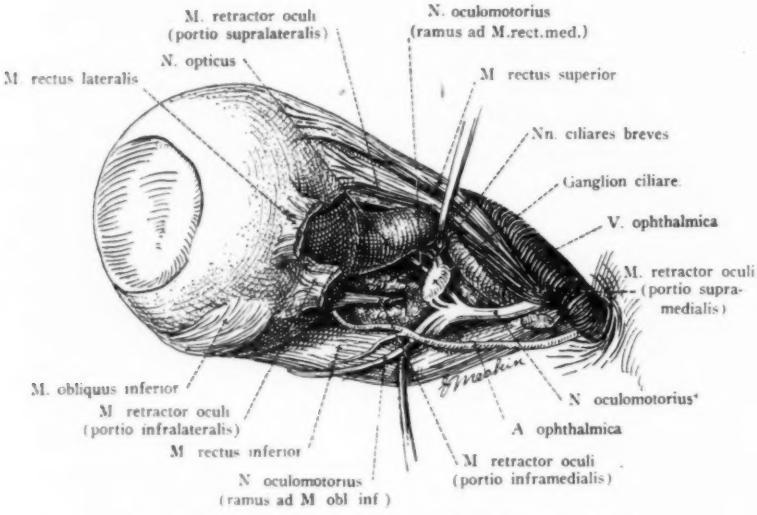

Fig. 1.—Dissection showing the ciliary ganglion of the dog.

fine-pointed scissors. From now on the field of operation must be kept absolutely clear of all oozing from very small vessels which may be unavoidably cut; and *in particular the ophthalmic vessels which will be constantly encountered.* The ophthalmic vein is situated just behind the point where the external rectus is severed and as it is comparatively large it is very liable to be cut if sufficient care be not taken. The ophthalmic artery on the other hand is situated farther forward; it crosses the inferior rectus muscle from a lateral position posteriorly to a point beneath the optic nerve about half way along the length of the muscle. More fatty tissue which is now met with must be dissected from the surfaces of the infra- and the supralateral quarters of the retractor oculi muscle. After doing this orient the eyeball properly by replacing the insertion of the external rectus muscle in its original lateral position. In order to enlarge the field of operation the superior rectus muscle may now be removed as was the external rectus. If this be done great care

must be taken to avoid cutting the ophthalmic vein which has an even closer relation to the point of origin of this muscle than to that of the external rectus. Detach the infralateral section of the retractor oculi muscle at its insertion on the eyeball and remove it, carefully dissecting it away from the structures immediately beneath it (the ophthalmic artery in particular) and cutting it off well back. Likewise remove the supralateral section of the retractor oculi muscle which also takes origin just beneath the ophthalmic vein. A nerve will be seen crossing the anterior half of the inferior rectus muscle from a medial to a lateral position and disappearing forward under the ventral end of the muscle. This is the branch of the inferior ramus of the oculomotor nerve which goes to the inferior oblique muscle. Follow this nerve backward and medially and the ophthalmic artery will be seen to cross it about half way along the dorsal surface of the inferior rectus muscle. Just behind the ophthalmic artery the minute branches of this nerve supplying the inferior rectus muscle may be seen. Continue following this branch medially and beneath the inframedial section of the retractor oculi muscle until it is seen to unite with another branch coming from the medial portion of the orbit from underneath the optic nerve. This is the branch of the inferior ramus of the oculomotor nerve which supplies the internal rectus muscle. By gently raising the inframedial section of the retractor oculi and the optic nerve together the ciliary ganglion may now be seen in the angle between these two nerves. From the ciliary ganglion the short ciliary nerves pass dorsally and somewhat medially to the optic nerve and follow it closely to the eyeball; consequently extreme care must be taken to avoid them in the final stage of the operation. This consists of removing the inframedial section of the retractor oculi muscle and the small amount of fatty tissue in its immediate neighborhood.

The electrodes used in stimulating the ganglion should be curved at their tips and applied from the direction of the ophthalmic artery, that is, laterally, by hooking them under the structure, while the optic nerve is held to one side. By carefully examining the dorsal surface of the optic nerve the long ciliary nerve may be seen lying in juxtaposition to it in the form of two or three very delicate filaments. These may be stimulated directly or through the cervical sympathetics. If the latter is done we have found it advantageous to separate the sympathetic strand from the vagus nerve just above the inferior cervical ganglion where they are not so intimately connected as higher in the neck. In some dogs, however, the sympathetic, which is always the smaller of the two and uniformly situated laterally, may be found more or less separate from the vagus at about the middle of the neck. Examination of the heart during stimulation will indicate whether any vagus fibers are involved.

The comparatively large field afforded by this method of exposure lends itself very well to the study of the function of the sympathetic and parasympathetic mechanisms involved. Drugs may be applied directly to the ciliary ganglion and their action controlled at will; or, on the other hand, the preganglionic or postganglionic fibers may be stimulated.

Finally, as pointed out by McGuigan (l. c.), the whole eye may be enucleated and the same structures stimulated as before. This is easily done by dissecting the organ away from all around the orbit, keeping close to the bone, and then cutting through the optic nerve and all of the structures surrounding it with a pair of fine-pointed curved scissors, the tips of which are inserted above the eyeball and posteriorly and downward as near the optic foramen as possible. Some distinguishing mark should be placed on the dorsal midline of the eyeball for the sake of properly orienting it after its removal. A little dissection, similar to that detailed above, suffices to disclose the ciliary ganglion and its associated nerves. Such a preparation, if kept in a shallow vessel on a pad of cotton soaked with normal saline solution and occasionally moistened, or, when not in actual use, covered by a similar pad, will keep in good condition and show contraction and dilatation of the pupil for half an hour or more.

THE VALUE OF THE ROSS-JONES TEST ON BLOODY SPINAL FLUID*

By S. M. Feinberg, M.D., Chicago, Ill.

ONE of the most important changes in the cerebrospinal fluid in a variety of pathologic conditions of the central nervous system is the increase in the globulin content. To determine this increase in globulin the majority of physicians and laboratory technicians employ the Ross-Jones test, this being perhaps the most useful, simple, and conservative. When a physician obtains spinal fluid, his first thought is to test for a pathologic increase in globulin, this usually giving him his first intimation as to whether the spinal fluid is normal or pathologic. Consequently, when anything interferes with the interpretation of the test so that the clinician cannot be sure in his own mind whether a positive or negative Ross-Jones test, in the particular specimen of fluid, is of any significance, then, one of the most valuable bits of information concerning that spinal fluid is lost.

This is precisely what happens when the spinal fluid is contaminated with blood due to trauma in performing the puncture; and this occurs more frequently than most of us are accustomed to believe. One authority† in his book on spinal fluid, says: "In doing any globulin test, one must make sure at the beginning, that the fluid is perfectly free of blood. Should there be any blood present in the fluid, the globulin test will be positive, even if no pathologic process exists." Other authorities express similar opinions. Consequently, we are led to believe that when one obtains traumatic blood in the spinal fluid the globulin tests should be absolutely disregarded.

However, I had frequently observed that the amount of blood in the spinal fluid, as determined by the number of erythrocytes per cubic milli-

*From the Cook County Hospital, Chicago, Ill.

†Levinson: Cerebrospinal Fluid, St. Louis, C. V. Mosby Co., p. 153.

meter, made a difference in the results of the globulin tests. And with this observation in mind, it occurred to me that it would be of some practical value to determine experimentally the amount of blood that must be present in the spinal fluid in order to give positive globulin tests, so that when subsequently a spinal fluid is obtained that contains traumatic blood, we may be able to tell, by counting the number of red blood cells per c.mm., whether it could affect the globulin tests. A series of experiments was done using normal spinal fluid, which on previous examination had been found to have a normal cell count and negative Ross-Jones test, and later found to have a negative Wassermann. In each experiment several tubes of spinal fluid from the same case were used, and to each tube was added a definite proportion of whole blood. The results are expressed in the number of erythrocytes per c.mm. rather than as the dilution of the blood, because, although the serum-globulin is the portion of the blood which affects the results, the number of red blood cells is, nevertheless, an expression of the total quantity of blood, and at the same time, it is comparable to the test for which it is intended. The experiments were made to conform as much as possible with the actual problem in mind; consequently, very little normal saline was used in the dilution of the blood.

The technic of each experiment was as follows: Several test tubes were filled with measured amounts of spinal fluid. To the first was added 1 c.c. of blood diluted a thousand times with normal saline. Starting with tube one successive dilutions of the blood were made in the tubes so that as a result, there was in the first tube the amount of blood necessary to give 1000 erythrocytes per c.mm. of the spinal fluid, and in the following tubes 750, 500, 333, 250, 125, and 62. The resulting mixtures were used for the globulin tests. After several trials with the Pandy test it was found to be unreliable and difficult to judge, and it was decided to use the Ross-Jones test, as this appeared to be satisfactory. Over .6 c.c. of the saturated ammonium sulphate was layered .6 c.c. of the spinal fluid containing blood, and the positive and negative results noted. The results are shown in Table I.

From this table it will be seen that the results are fairly constant with several normal spinal fluids from different cases. It is evident that in none

TABLE I

THE ROSS-JONES TEST IN SPINAL FLUIDS CONTAINING BLOOD

SPINAL FLUIDS	1 to 5000 dilution or 1000 r. b. c. per c. mm.	750	500	333	250	125	62
Alcoholic Psychosis	+	+	trace	0	0	0	0
Neurasthenia	+	+	+	?	0	0	0
Senility	+	+	+	?	0	0	0
Alcoholic Psychosis	+	+	+	0	0	0	0
Hysteria	+	+	trace	0	0	0	0
Senility	+	+	trace	0	0	0	0
Macroscopic appearance	turbid	turbid	cloudy	opal-escence	slight opal-escence	?	clear

of the cases was there a positive Ross-Jones test with the amount of blood necessary to give 250 erythrocytes per c.mm.; that in the dilution of 333 and 500 the results were variable; and that in the dilution of 750 cells or over the Ross-Jones test was uniformly positive. Following these determinations the results were applied to cases with spinal fluids which were actually bloody because of trauma and the results appeared to be of clinical value. It was also noted that a turbidity of the spinal fluid caused by the red blood cells was recognizable at a dilution of 1 to 20,000 or 250 cells per c.mm.; in other words, at a dilution too high to give a positive Ross-Jones test.

CONCLUSIONS

From the above it seems evident that a definite amount of blood must be present in the spinal fluid before a positive globulin test is obtained; that this amount is the concentration necessary to give in the neighborhood of a little over 300 cells per c.mm.; that when one obtains traumatic blood in the spinal fluid with a positive Ross-Jones test, if by determining the amount of blood present it is found to be below the necessary limit as here determined, the fluid is undoubtedly pathologic.

THE TRAINING AND PROPER RECOGNITION OF THE LABORATORY TECHNICIAN

By R. B. H. Gradwohl, M.D., St. Louis, Mo.

DURING the past ten years there has been a remarkable development of the so called clinical laboratory. The number has become so large that it is practically impossible to man these institutions with graduate medical personnel. The reason for this is obvious: Most young physicians from whose ranks special workers are usually drawn prefer other lines than laboratory work for the development of their special faculties. Only a small number deliberately select the laboratory as a permanent career. Some enter it temporarily as a stepping stone to something else, usually internal medicine or surgery. For the most part they do not enter this field at all even temporarily. We are therefore confronted with this situation: a rapidly growing appreciation on the part of the profession for laboratory help in diagnosis; the establishment of more and more so called "commercial laboratories", which by the way is a most unwarranted and objectionable designation, the better equipment and organization of laboratories in hospitals which are taking part in the standardization plan now under way; the development of private laboratories in the offices of individuals or groups of individuals practising internal medicine or surgery; the rapid growth of public health laboratories, municipal, state and national: all this has created a demand for workers which the medical schools alone could hardly supply even were their graduates willing to enter upon this career. Inasmuch as the medical school graduates cannot and will not fill this demand, we directors

have rejoiced in the fact that we have been helped materially in our everyday routine labors by the advent of the laboratory "technician."

And who and what is a "laboratory technician"? He or she, as is more commonly the case, is a faithful ally and helper for the laboratory chief or his assistants. I shall refer to "her", as the number of male technicians is so much smaller than the female. I do not know when or where the term arose. I recall in my younger days abroad the remarkable feats to my inexperienced eyes of the so called "Diener" of the German laboratories. We might consider therefore the American laboratory technician as a transmigratory and glorified representation of the "Laboratory Diener" of the olden days. It is sufficient to say that at the present time there is a group of women (and an occasional man) who have seriously entered upon the career of laboratory technical work. Their preliminary training has been as variegated as that of the medical graduate of the pre-standard days of medical school requirements. Some have just "picked up" technic around some doctor's office or laboratory, and have little or no education and possibly have begun their medical work in the humble capacity of office attendant. Others have had a high school education and have then secured a position as a helper in some hospital or public health laboratory. More recently a number of college-bred women have prepared themselves for the work by taking up science courses and then the special medical studies of clinical pathology, the recognition of pathogenic bacteria, serology and blood chemistry. In short, just as the demand has grown, so has the supply been fairly made adequate by this entry into our ranks of these women workers. And as a better appreciation of their labors has been manifested, so a better prepared class have entered the ranks. There was a time when in referring to a piece of work, some physicians might disdainfully say, "this was done by some technician," and thereby indicate his scorn of the result and findings— that time seems happily about to pass for the reason that the despised technician is now doing excellent and dependable laboratory tests. Her reliability is in direct proportion to her training, her experience and her laboratory direction just as is the case with any other special worker in medicine. I have known women technicians who have gone the length and breadth of the land, surveying and watching the work of their masters, taking special courses with this or that man, returning to their laboratory work much improved in technic, just as is done by all workers in medicine who wish to keep abreast of the times. There is rank injustice therefore in the remarks of those who attempt to belittle the work of technicians. There are technicians and technicians, just as there are doctors and doctors. The well-trained, honest and reliable technician is of inestimable help to us and we cannot get along without her. The main question to be settled regarding the work of the laboratory technician is just how far these people ought to go in making a laboratory test and its interpretation. In my experience I have seen a number of technicians who are sufficiently well trained to conduct all of the standard tests of a laboratory, to-wit: clinical pathology, serology, blood and urine chemistry and even the later work of the laboratory designated under the title "Basal Metabolism." Some of these workers are sufficiently intelligent and well trained to procure the specimen properly from the patient and carry out every step of the procedure. There are others who

are not so well trained and their work must be closely supervised and controlled by the laboratory director, usually a man with a medical degree. The point I want to emphasize is that the *properly educated* technician is fully able to carry out the routine laboratory tests required by physicians in their everyday practice. I do not by any means wish to say that these people are qualified to interpret all of their findings. After all, the manner of interpreting laboratory findings is one that properly belongs to the medical trained laboratory worker or the laboratory trained clinical worker. It also is to be understood that my remarks concerning the value of a technician in the laboratory field, in so far as tissue diagnosis is concerned, is that they are qualified to make sections but not to make diagnoses. It is unnecessary, of course, to emphasize the necessity of a long training in pathology to equip one to be a reliable tissue pathologist.

I have found after many years of practical experience in this line that, in the main, women workers are essentially honest and conscientious, the possessors therefore of the two fundamental qualities in laboratory investigation. Leaving aside the relative dependability of the male versus female technician, we are face to face with this situation; owing to the great demand for laboratory work and the scarcity of medical talent to perform all of it, we must look elsewhere for help than to the medical school product. That "elsewhere" is the laboratory technician group. Since their work is highly satisfactory, it behooves us as medical men to whom these people come for their employment to lay down certain standards both for preliminary training and technical education which we consider fitting requirements for entrance upon serious labor of this kind. Medical examining boards reinforced by the remarkable self-purifying process undertaken in the past twenty years by the medical schools, urged and helped by the Council on Medical Education of the American Medical Association, have brought order out of chaos in American medicine and are producing a medical product called a "physician" who is really properly trained to pursue his calling with the minimum of danger to the individual citizen who from time to time is designated the "patient". The medical school problems are therefore well nigh solved; not so with the technician question.

We have no state medical examining boards for technicians. Neither have we organized schools for their training. Possibly the only well-known school in America was that initiated a few years ago by Doctor John Kolmer of the University of Pennsylvania. This we understand has been lately abandoned. These people have been given training in a few postgraduate medical schools but no school so far as I can ascertain has been conducted for the purpose of giving a woman or man a complete technical course in the subject of clinical pathology or clinical laboratory diagnosis. Nor does there exist so far as I can ascertain, any board, court or tribunal which passes upon the qualifications of these so-called technical workers. During the late war their services were much sought after for Government work. We are informed that the Army employed 398 technicians during the period of the war. Their services were eminently satisfactory according to the Army authorities. The United States Public Health Service also uses the services of technicians. They secure posi-

tions after taking the civil service examination. There are various grades of laboratory workers used in the public health services, namely; Laboratory Assistant, Junior Bacteriologist, and Bacteriological Technician. Dr. G. W. McCoy, Director of Hygienic Laboratory, Washington, D. C., states at the present time they are using about 15 technicians in that institution.

RECOMMENDATIONS

1. The establishment of schools under proper direction for the training of these persons.

2. The recognition of the technician of experience by some kind of board with authority to pass upon qualifications and give a certificate of merit to those who deserve it.

3. The organization by the laboratory workers *themselves* of an association for the betterment of their professional status, the purging of incompetent workers from their midst, and the matter of propaganda throughout the land among women's organizations for the purpose of enlisting the interest of women in this work, and the entrance of properly trained persons into technicians' schools.

If you will note the number of advertisements each week in the columns of the leading journals for physicians or hospitals or laboratories asking for technical help, you will gain in a measure some idea of the insistent demand for technicians. If you will also realize how difficult it is to engage the services of a technician at a distance, who has by virtue of the present haphazard system, no certificate except the letters of recommendation of her past employers, you will likewise appreciate the difficulty for a laboratory director to engage these people without a try-out which at times is very expensive and often impossible. How much better would it be were the prospective technician able to say, "I hold a certificate of ability from this or that medical board," thus simplifying the process of employment considerably. The difficulties of the doctor in California employing by letter the technician in Maine would thus be materially eliminated. At times I am overwhelmed by the number of requests for technician assistance. And even when I manage to get the address of some technician through the medium usually of classified "Want" columns, there ensues a long series of letter-writing before the two interested parties can be brought together. This I contend might well be eliminated by some sort of board, possibly some volunteer examinations conducted by a representative Bacteriologist in each one of our metropolitan cities.

The Journal of Laboratory and Clinical Medicine

VOL. VI. AUGUST, 1921 No. 11

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Ann Arbor, Mich.

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EDITORIALS

The Hemoglobin Content of the Blood

THE estimation of hemoglobin was apparently the first chemical determination in the blood to find extensive clinical application. It seems unfortunate that most of the estimations recorded should have been made employing an empirical scale with 100 as the normal, especially since the 100 is somewhat of a variable factor with different methods owing to different standardizations. Of the older clinical instruments the Fleichl-Miescher alone is standardized to give the actual percentage of hemoglobin. Sahli[1] states that in his original instrument the 100 was designed to correspond with an actual hemoglobin percentage of 17.2 per cent. Haldane[2] employs as a standard of 100 a 1 per cent solution of a blood having an oxygen capacity of 18.5 per cent. Such a blood contains approximately 14 grams of hemoglobin per 100 c.c., a quantity rather below that found in normal adult blood by most workers. Haldane states that with normal women he found an average oxygen capacity of 16.5 per cent and with children 16.1 per cent, thus necessitating a different standard of 100 for women and children.

The hemoglobin content of the blood varies widely not only in disease but also during different age periods, as recently pointed out by Williamson.[3]

For these reasons it is much more logical to record hemoglobin as we do other blood determinations in grams per 100 c.c., or actual per cent.

Williamson has taken great pains to definitely establish the normal hemoglobin content of human blood of both males and females for the different age periods. His figures for these different periods were made with the accurate spectrophotometric method and were the average values found on fifteen or more individuals. It was ascertained that during the first two weeks of life the hemoglobin content exceeds 20 per cent, but then drops rather abruptly about the third month to below 14 per cent and does not pass this figure until the tenth year. During the adult period of life in both sexes (from 16 to 70 years) the hemoglobin maintains a fairly constant level of slightly above 16 per cent. From the third month to the fifteenth year the values obtained in the female appear to slightly exceed the male, although from 16 to 60 the reverse is true, the hemoglobin of the female averaging close to 15.5 per cent, while in the male it reaches nearly 17 per cent.

A few observations with the same method were reported a little earlier by Meyer and Butterfield.[4] An average of seven normal men gave 16.6 per cent hemoglobin, while the findings for six normal women were 15.2 per cent. Since their observations were made in Germany, and agree closely with those of Williamson made in this country, it may be assumed that, for ordinary altitudes, the hemoglobin content of human blood for the adult period of life is relatively constant, being about 1.5 per cent higher in the male than in the female.

Bock[5] has recently made the very interesting observation that the blood plasma volume is very constant under normal and a great variety of pathologic conditions, forming about 5 per cent of the body weight. The average volume found by Bock for the whole blood of normal individuals was 8.2 per cent, while in his series of pernicious anemia cases it amounted to 5.7 per cent and in polycythemia to 13.7 per cent. Since the plasma volume is practically constant, the differences in the blood volume in these two conditions is obviously dependent upon differences in cell content, chiefly in that of the hemoglobin carrying red cells. Bock's normal cases showed a hemoglobin content (recalculated) of 16.4 per cent, whereas in pernicious anemia it was 6.5 per cent and in polycythemia 22.5 per cent.

Normally the total solids of the blood amount to about 21 per cent, and of this fully 16 per cent is accounted for by the hemoglobin. This being the case, hemoglobin is by far the most important variant in pathologic bloods. Consequently its estimation should prove of considerable value as a part of routine chemical blood analyses.

Excepting such blood diseases as pernicious anemia and chlorosis, where the hemoglobin content of the cells may be increased or decreased, respectively, thus giving rise to a high or low so-called "color index," the hemoglobin parallels fairly closely the number of red cells, and, consequently, furnishes little added information. For this reason, if one excepts the conditions mentioned, a hemoglobin estimation might more logically supplement a chemical blood analysis than a blood count.

Owing to the increased number of colorimetric methods now in common use, especially in connection with the chemical analysis of blood, a standard colorimeter (Duboscq, Kober, Bock-Benedict) has become a part of the necessary equipment of every clinical laboratory at the present time. This being the case, there is no reason why the same instrument should not be utilized for the estimation of hemoglobin. Adaptations of the Hoppe-Seyler-Haldane carboxyhemoglobin method and the acid hematin method of Sahli have already been made to these instruments. Obviously the accuracy is far greater than with the microcolorimeters ordinarily employed for hemoglobin estimation.

In the past one of the greatest difficulties in the way of making an accurate colorimetric hemoglobin estimation has been to secure a correct standard. This is now in a fair way of solution owing to the development by Van Slyke[6] of a simple gasometric method of determining the oxygen capacity of the blood. Van Slyke employs the same gas burette as in his CO_2-combining power estimation, and this instrument is now in nearly every clinical laboratory. As Smith, Dawson, and Cohen[7] have pointed out, probably some additional factors need to be considered in making an absolutely accurate calculation, but this method should ultimately furnish the basis of quickly obtaining a correct hemoglobin standard, since the oxygen capacity of blood is dependent upon its hemoglobin content. We have long known from the observations of Hüfner that one part of hemoglobin combines with 1.34 volumes of oxygen.

Another method of checking hemoglobin standards has been suggested by Berman,[8] viz., the estimation of its iron content. He has described[9] a method of estimating iron in blood which may be utilized for this purpose.

Palmer[10] has recently described a modification of the Haldane carboxyhemoglobin method which may readily be made on the oxalated bloods employed in chemical analyses. He states that with this method estimations may be made in two minutes' time to an accuracy within about one per cent. The quantity of blood required (0.05 c.c.) is so small that satisfactory results may also be obtained when the usual clinical method of obtaining small amounts of blood by pricking the ear or finger tip is carried out with proper care.

When care is taken several workers have recently shown that satisfactory results may be obtained with the Sahli acid hematin method. Three serious criticisms may be made of this method as it is ordinarily carried out clinically: The standards are often inaccurate, the dilution colorimeter employed is too small to yield accurate results, and sufficient time is not generally allowed for the color to develop. Smith and Cohen[11] have shown, however, that with proper care quite as accurate results may be obtained with this as with the Palmer method. Robscheit[12] has likewise pointed out that when at least one hour is allowed for the color to develop, she was able to obtain as consistent results as with the Palmer technic. Berman[8] has also shown that heating for one minute will accomplish the same purpose. As a standard Newcomber[13] employs colored glass plates, which may be placed in the cups of the colorimeters. Although these glass standards are obviously permanent,

they have the disadvantage that each plate requires careful standardization and does not match perfectly the acid hematin in all concentrations.

It is suggested that the time-honored hemoglobin test would yield results of much greater clinical value if more attention were given to accurate standards and methods, and the results then computed to give the actual percentage of hemoglobin rather than the relation to an indefinite normal in parts per 100.

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—*V. C. M.*

Influenza and Tuberculosis—A Postscript

A QUESTION is never trite until it is settled. Despite the formidable volume of literature which has accumulated since the influenza pandemic, dealing with the relationship between this disease and tuberculosis, the views expressed are still various and often contradictory. Much of this literature was analyzed by Vaughan[1] in a very thorough and critical article which appeared in this journal last autumn. What we have to say may be regarded as a footnote to his editorial.

With the advantage of a trained scientific mind, Vaughan was able to recognize the imperfect quality of the data available, and the opportunities for fallacy, and his conclusions were cautiously expressed. They were, briefly, that different observations on this subject are certain to vary, because the conditions are not constant; that phthisical patients in the mass are relatively insusceptible to influenza; but that they frequently do contract it, and when they do, it hastens their death from tuberculosis in many instances; finally, that latent tuberculosis may be activated by influenza.

Recently Fishberg[2] has emphatically reiterated his radical views on the subject. He writes, "We are safe in concluding that influenza has no etiologic relationship to tuberculosis." This opinion is based chiefly on the facts that the tuberculosis morbidity, judged by the demand for sanatorium treatment, and the tuberculosis mortality, judged by vital statistics, have not only shown no increase since the pandemic, but have actually decreased.

This test of morbidity, if applied to a limited section of the country, involves obvious possibilities of error. According to Fishberg, none of the sanatoria in New York are filled. In Colorado, as far as our observation goes, they

are filled, but show no abnormal congestion. The south-western states, on the other hand, are said to be crowded with tuberculous invalids; but it may be that few of these patients come from the Atlantic seaboard, and that Fishberg's observation does represent a real decrease.

Mortality statistics are more reliable, and there is no doubt that both in America and Europe, wherever nutrition is adequate, these have shown a decided decrease in the tuberculosis death rate in the last two years. Even here, however, there is danger in hasty conclusions. It has been pointed out[3] that a disease like chronic nephritis, even if caused by influenza, will not appear in the mortality statistics till several years later, and the same is clearly true of tuberculosis.

We, as physicians, are imbued with the text book dogma that influenza is among the chief excitants of tuberculosis, and it is difficult to rid ourselves of it. Once free of it, there is a temptation to run ahead of the evidence to the opposite extreme. For this reason it seemed desirable to consult an expert statistician, and we addressed these questions to Vice-President Frankel of the Metropolitan Life Insurance Company:

1. What is the average duration of life in fatal cases of tuberculosis, after the first definite signs of activity?

2. What, in your experience, has been the effect of the influenza epidemic in America upon the tuberculosis death rate?

His reply,[4] based chiefly upon data from twelve million policy holders in the Industrial Department of his Company, was as follows:

"1. This question is as yet incapable of answer. To answer this question, it would be necessary for a considerable number of years to trace the life history of patients from their earliest symptoms of tuberculosis until death. As you can see, that is a considerable undertaking and no one has thought of doing it except for very limited groups in certain sanatoria. As you probably know, this defect in the statistical situation is being remedied by the National Tuberculosis Association, which, at the suggestion of Dr. Dublin, has recently put into operation a complete plan of record keeping for all sanatoria of the country that will cooperate. This will mean that hereafter records will be available of the after-life history of the patients. Ultimately, your question will be capable of solution.

"2. It is a very interesting thing to watch the effect of the epidemic on tuberculosis. The death rate from tuberculosis rose almost at once. The rate for tuberculosis of the lungs went up to 149 per 100,000, as compared with 130 in the same quarter of the preceeding year. Thereupon, the rate began to fall and the figure for tuberculosis of the lungs for the whole year was one of the lowest on record, 141.6; in 1920, the rate still further declined, reaching the unprecedented figure of 121.5. I would, therefore, say that the effect of the influenza epidemic on tuberculosis was very slight indeed. In fact, it was limited to the immediate period of the epidemic itself. After the epidemic, the tuberculosis mortality situation seems to have been greatly improved. It is, of course, possible that we are dealing with artifacts, that is, many of those who died of influenza, died in fact, of advanced tuberculosis

hastened by the influenza process. Many such cases undoubtedly occurred and were not certified as tuberculosis. This would, of course, explain some of the remarkable drop in the tuberculosis rate in subsequent periods. It is still an open question."

Another chance of error in interpreting the available figures lies in the fact that the greatest number of deaths in the epidemic fell in an age period —20 to 35—in which the death rate from tuberculosis is always high (3.5). Whether this has been of any importance in reducing the number of candidates for fatal tuberculosis we have no means of knowing—probably not, unless those who died constituted a susceptible group, but this may be the case.

It is clear that we have not, and shall not have for several years, adequate data to determine with certainty the effect of the 1918-1920 influenza epidemic upon the tuberculosis situation; and when this is settled, we shall not be certain that our conclusions will apply to the next epidemic, or to the endemic disease—so dark and foggy a field is medical science. But it already seems safe to infer, from the lack of evidence to the contrary, that the effect of the epidemic on tuberculosis was far less than we feared.

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—*G. B. W. (C. T. R.)*

Negative Wassermann Reactions in Syphilis

THE serologic study of syphilis has become so extensive that in large medical hospitals and clinics the Wassermann reaction has become practically routine. Coincident with the development of routine tests for syphilis, clinicians have come to rely more and more upon the laboratory for their diagnosis and have neglected the methods of physical examination and analytic diagnosis. In the past when syphilis was suspected a thorough search was immediately instituted for any and all signs or stigmata suggesting this disease. How much easier it is to await the laboratory report and thereupon establish or eliminate syphilitic infection!

Not only is routine laboratory technic not infallible, but furthermore, there are without doubt not a few cases of syphilis with truly negative Wassermann reactions. These cases will be misdiagnosed if too great reliance is placed upon the laboratory examination. Pride in one's ability to recognize disease should impel one to make a thorough examination and to establish the tentative diagnosis, even before receipt of the laboratory report. If the report is then found to be at variance, it is still possible that the laboratory is in

error. It is then that judgment, that rare virtue, so necessary to the diagnostician, becomes of paramount importance.

Perhaps the condition most frequently met with, in which the patient although actively syphilitic shows a negative blood Wassermann, is chronic advanced syphilis of the central nervous system. Here the spinal fluid will usually show a positive reaction.

Marcel Pinard describes a case with multiple, painful exostoses without signs of syphilis and with a negative Wassermann and Hecht reaction, but which subsequent events showed to be a case of heredosyphilitic infection. The initial symptoms appeared at the age of 22, following trauma. As the exostoses became more numerous, syphilis was suspected and the blood was tested and found to be negative. The spinal fluid Wassermann was also negative. Under antisyphilitic treatment the symptoms rapidly disappeared.

The same author has recently reported an even more interesting case. A pregnant woman of 35, with headaches, facial paralysis and albuminuria presented herself for diagnosis. Syphilis was suspected and a Wassermann was done on three occasions, and by three different serologists, each time with negative results. The woman at term gave birth to an infant showing all of the classical signs of congenital syphilis, with skin eruption, snuffles, etc.

Two children from a previous marriage were examined and in both were found the characteristic signs of heredosyphilis.

The woman had suspected syphilis before her second marriage and had consulted a physician who made blood tests and informed her that she was "une malade imaginaire." The patient remarried, with medical sanction, and continued to produce hereditarily syphilitic children.

Pinard draws attention to the unfortunate circumstances resulting from a medical attitude of mind which depends entirely upon the result of the Wassermann reaction for guidance, and emphasizes the importance of searching for evidences of syphilis among other members of the family, a search which often furnishes information which the serologic reaction withholds.

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—*W. T. V.*

Two Recent Papers on Pellagra

WHAT is the etiologic agent in pellagra? Is it due to an infection, or is it a nutritional disease, or do both of these agents play a part in its causation? These are questions that remain unanswered and still furnish abundant opportunity for argumentation. Among American students of this disease, MacNeal and Jobling insist that pellagra is an infectious disease; on the other hand there are Goldberger and his assistants who claim that it is a nutritional disease. Some years ago Goldberger made an experiment on eleven men in the Mississippi State Penitentiary. They were fed for some months

upon an unbalanced diet in which carbohydrates greatly and abnormally predominated. In six of these eleven patients an eruption said to be typical of pellagra developed. There has always been and still remains some question about the accuracy of this diagnosis. The result was not entirely satisfactory; it was not clear cut one way or the other. There is one thing, however, that Goldberger has demonstrated, apparently beyond question, and that is that proper feeding with an abundance of animal food both prevents and cures pellagra. The demonstration made at the great insane asylum at Milledgeville, Ga., seems to offer no other interpretation.

MacNeal[1] gives a very satisfactory review of the present state of the pellagra discussion. He holds that both nutrition and infection are essential in the causation of this disease. He points out that among 62,119 whites in Spartanburg County, S. C., in October, 1914, there were 1,027 pellagrins, an incidence rate of 165 per 10,000. At the same time among a negro population of 28,507 there were only 153 pellagrins, an incidence rate of 54 per 10,000. He claims that, notwithstanding the fact that the negroes live in greater poverty and have less abundant and less varied food, the incidence of the disease among them is much smaller than among the whites. Furthermore, he shows that most of the negroes who develop the disease are those who come into most intimate contact with the whites, serving as houseservants and in other relatively close relations. While pellagra is less prevalent among the negroes, it causes among them a higher death rate than among the whites. MacNeal apparently advances these statements with the idea of reinforcing his claim that the disease is infectious. Like Goldberger's experiment in inducing the disease by change in diet, we think that impartial readers will come to the conclusion that MacNeal's illustration is likewise not very convincing. It is just as easy to suppose racial differences in nutritional diseases as in infectious diseases. MacNeal, just before the war, was carrying on an experiment near Spartanburg, S. C., which was interrupted by his military service, but so far as it went MacNeal was inclined to believe that improved sanitation in a cotton village did much to reduce the prevalence of pellagra. Goldberger, studying the same village a year or two later, came to the conclusion that the improved sanitation had had no effect upon the incidence of pellagra. Studies along this line must be carried further. It is quite evident to the outsider that the contestants concerning the causation of pellagra are drawing nearer together. In the present paper MacNeal, in speaking of prophylaxis, says that the measures employed must be grouped into two classes; (1) those which enhance the individual resistance to the disease, and (2) those which exclude or diminish the opportunity for the introduction of the causative factor; in other words, this author evidently believes that the infecting agent causes the disease only among those who are nutritionally below par. On the other hand, Goldberger does not deny the possibility of infection, though he quite justifiably regards this as very remote. He and his assistants have inoculated themselves with every possible secretion and excretion of pellagrins and have not induced the disease. It might have been otherwise, how-

[1]Am. Jour. Med. Sc., 1921, clxi, 470.

ever, had they reduced their resistance before these inoculations were made, by living upon an unbalanced diet. The importance of nutrition in many of the infectious diseases is being more fully appreciated, and we are attaching to it more importance that we did. Whatever may be the ultimate verdict as to the etiologic agents in this disease, it is quite certain that by a properly balanced diet primary cases may be avoided and recurrences may be prevented.

The second paper on pellagra is by Sullivan, Stanton and Dawson[2] and concerns itself solely with the question of metabolism in pellagrins. Their results are substantially as follows: (1) Mineral metabolism as measured by the P_2O_5 elimination is low, even when a generous diet with an abundance of milk is given. (2) Putrefactive processes in the intestines are abnormally high. (3) Albumin and casts are found in the urine in about fifty per cent of cases, although there may be pellagra without change in the kidney. (4) The total nitrogen excretion is low; especially is this true of the urea nitrogen, suggesting insufficient activity of the liver. (5) The ratio for ammonia nitrogen and undetermined nitrogen is high. (6) The excretion of uric acid and creatinin is low. (7) The utilization of protein food, even after several weeks on a remedial diet, is subnormal. (8) After a month or more on the curative diet, urinary ingredients rise to approximately normal amounts. This is especially true of urea which rises to the normal, while the ammonia ratio falls to the normal. —*V. C. V.*

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